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SPORULATION BY PIRICULARIA ORYZAE[1]

B. W. Henry and A. L. Andersen[2]

(Accepted for publication September 3, 1947)

INTRODUCTION

During research on the relation of environmental factors to the development of the "blast" disease of rice caused by *Piricularia oryzae* Cav. (1), conidia were needed in quantity for use as inoculum in the various experiments. It was soon evident that sporulation by *P. oryzae* is a process distinct from mycelial growth in many respects. A certain amount of vegetative growth is of course prerequisite to the formation of conidia, but did not always lead to sporulation. Miller (13) has recently recorded similar findings with other plant pathogenic fungi. Tochinai and Shimamura (19), Konishi (8), and Aoki (2), reported physiologic specialization in *P. oryzae* using the degree of sporulation in culture as one characteristic. The present paper describes the process of spore formation by *P. oryzae*, the effects of some factors upon sporulation, and the relation of sporulation to macroscopic characters.

MATERIALS AND METHODS

Subcultures of a strain of *Piricularia oryzae* furnished by Dr. E. C. Tullis,[3] or of its reisolates obtained by plant passage, were used in all the reported experiments. Temperature effects on sporulation were studied with the aid of incubators adjusted to $\pm 0.5°$ C. The standard substrates used were 2 per cent rice-polish agar, and a 1:1 mixture of oats and Hegari sorghum seed plus water equal to 1.15 times the dry weight of the mixture. The comparison of these with other substrates is discussed later. Culture vessels for the whole-seed substrates were of three types. One consisted of 3 wire-screen trays in an enameled photographic pan $16\frac{1}{2} \times 9\frac{1}{2} \times 1\frac{1}{2}$ inches, with another pan inverted over the top. A $\frac{1}{2}$-inch opening was left at one end by supporting the top pan on cork stoppers. The trays, each $13\frac{1}{2} \times 7\frac{1}{2}$ inches, were tiered inside the resulting semi-closed chamber by supporting the corners of each tray on cork stoppers coated with paraffin. About $\frac{1}{4}$ inch of sterile water was kept in the bottom pan. To forestall contamination by air-borne organisms, the substrate samples were sterilized, inoculated, and incubated for 3 or 4 days in quart milk bottles plugged with cotton, prior to placing them on the trays. The photographic-pan type of vessel was used

[1] Work conducted at Camp Detrick, Frederick, Md., from February, 1944 to September, 1946.

[2] The authors gratefully acknowledge the technical assistance of B. M. Goodloe and T. L. Morgan.

[3] Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

for some of the initial studies on the different grain substrates and for the production of quantities of spore inoculum. It was soon supplanted, however, by the other types.

The second type of vessel was a galvanized-iron chamber $18 \times 12 \times 16$ inches with a sliding panel door and contained ten removable copper-screen trays (Fig. 1, A). The entire chamber and aeration attachments (exclusive of the manometer) were autoclaved for one hour at 20 pounds steam pres-

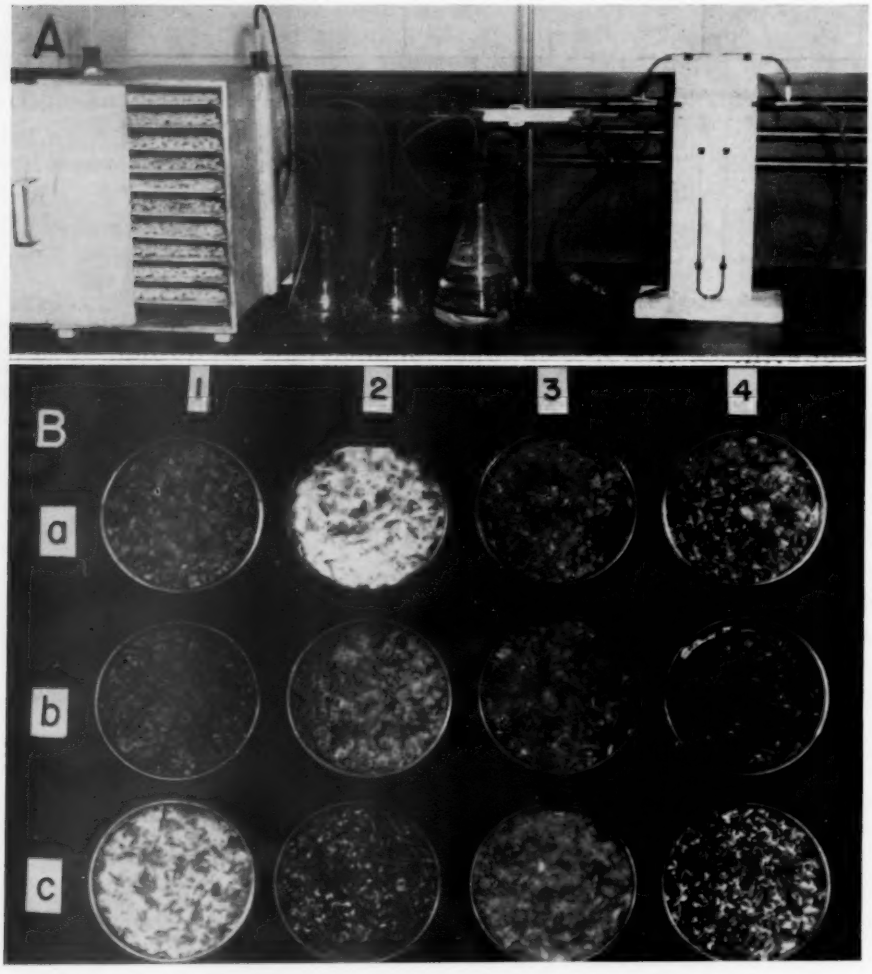

FIG. 1. A. Growth chamber for studying sporulation on whole-seed substrates. B. Petri-dish cultures of *Piricularia oryzae* on oats-sorghum substrate showing variation in macroscopic characters. Cultures b–1 and b–3 produced conidia in abundance; cultures a–2 and c–1 were nearly sterile; others were intermediate.

sure. One 200-gm. or two 100-gm. dry wt. samples of substrate were used per tray. As with the photographic-pan type of vessel, the seeded substrate was incubated in quart milk bottles prior to placing it on the trays. The chamber was supplied with filtered, humidified air at a controlled rate meas-

ured by the manometer in the air line. To increase the number of samples that could be tested, the chamber was also used as an incubator for Petri-dish cultures. Fifteen-gm. dry wt. samples of substrate were sterilized, inoculated, and incubated for 2 to 3 days in 9-cm. Petri dishes. Twelve dishes were then placed on each tray, the lids removed, and the incubation period completed. It was thus possible to test 120 samples at one time in the chamber. Since four replications were desirable, 30 isolates or 30 substrates could be tested simultaneously. This type of vessel was used for studies on the different grain substrates, the different isolates of *Piricularia oryzae,* the effect of inoculum concentration, and for producing spore inoculum in quantity.

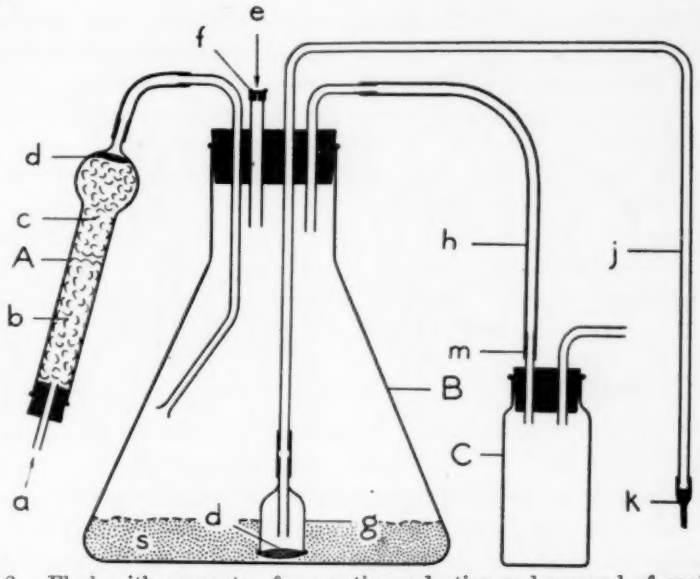

FIG. 2. Flask with apparatus for aseptic production and removal of conidia. A. Air filter. B. The 3–l. Fernbach flask. C. Air trap. a. Air inlet. b. Glass wool. c. Absorbent cotton. d. Copper screen. e. Inoculation tube. f. Serum bottle cap. g. Filler tube. h. Air outlet and water inlet. j. Suspension exit tube. k. Rubber policeman. m. Rubber hose connection to facilitate the removal of air trap (C) prior to the introduction of water through (h) at time of harvest. s. Culture on whole-seed substrate.

A third type of culture vessel, for studies on whole-seed substrates, *e.g.,* rate of aeration, period of incubation, etc., was a large-mouth, 3-l. Fernbach flask equipped for forced aeration, for the aseptic introduction of sterile water, and for the aseptic removal of the spore suspension (Fig. 2). A 200-gm. dry wt. sample of substrate was used. The spores were put into suspension by shaking the flask in a rotary manner, and the suspension was forced out through the "filler tube" by slight air pressure. For experiments in which the spores did not need to be harvested aseptically, only that equipment needed for forced aeration was employed.

Spore suspensions for seeding all cultures were obtained by adding sterile water to 5- to 7-day-old agar cultures in tubes and lightly scraping the surface with a wire loop. Agar media in test tubes were seeded by spreading

a 5-mm. loopful of the suspension over the surface of the agar. Whole-seed substrates were seeded by aseptically applying a given amount of the suspension from a hypodermic syringe and distributing it by gently rotating the culture vessel. Spore yields and spore density of inocula were determined with the aid of a "Howard Mold Counting Chamber, modified type" (Arthur H. Thomas Co., Philadelphia, Pa.).

THE PROCESS OF SPORE FORMATION

The sporulation process of *Piricularia oryzae* was studied in detail on water and rice-polish agars using hanging-drop cultures, agar films on microscope slides, and cleared agars in Petri dishes. The conidia were obclavate to pyriform, pale olive to colorless, usually 3-celled, and approximately 8–9 × 20–26 microns. They were formed on conidiophores morphologically similar to the vegetative hyphae. At first a single conidium formed at the apex of a conidiophore (Fig. 3, A). Later a branch grew from the conidiophore a few microns below the point of attachment of the first spore, and another conidium was formed at its apex (Fig. 3, B, C, and D). A second branch appeared a few microns below the point of attachment of the second spore and the third conidium was formed (Fig. 3, E). This process of sympodial branching continued; as many as 9 conidia were observed on 1 conidiophore. General observations of sporulation on individual seeds of oats and sorghum, and on host lesions confirmed the detailed studies. These data are in general accord with those of Aoki (2), and indicate that the process of spore formation by *P. oryzae* in artificial culture is like that occurring on the host.

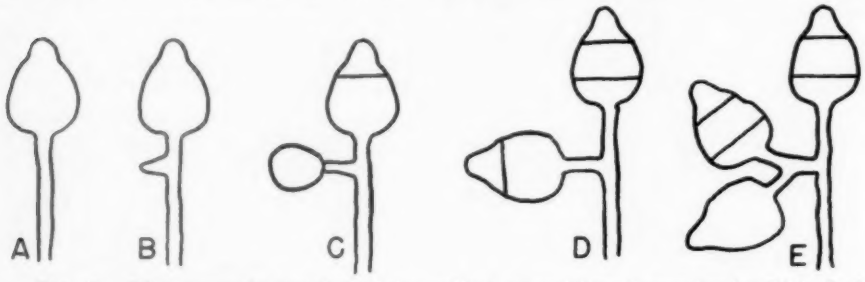

FIG. 3. Diagrammatic sketches of the method of conidium formation by *Piricularia oryzae*. Details are given in the text.

FACTORS AFFECTING SPORULATION

Concentration of Inoculum

In studying the growth and metabolism of fungi in culture, it is usually considered that, within quite wide limits, the concentration of spore inoculum is not a determining factor (11, 15). The incubation period required for the production of certain metabolic products by fungi in liquid culture has been reportedly shortened by using heavier spore inoculum (5, 22). No reported work on the effect of this factor on conidia production has come to the authors' attention. In order to standardize the procedure for further

experiments, the effect of inoculum concentration on spore yield was determined for both the agar and the whole-seed substrates.

Rice-polish-agar slants were seeded with suspensions having 0.01, 0.05, 0.1, 0.5, 1.0, 2.0, and 4.0 million spores per ml. Spore yields were determined after 7 days' incubation at 25° C. The inoculum containing 0.01 million spores per ml. gave a yield of 14 million spores per tube, and all higher concentrations yielded 20 to 24 million spores per tube, except the concentration of 2.0 million per ml. which gave 29 million per tube. Further experiments failed to show the peak with the 2.0 million per ml. concentration. It was concluded that a 5-mm. loopful of a suspension of 0.05 million or more spores per ml. was optimum for seeding the agar slants.

One-hundred-gram units of oats-sorghum substrate in quart milk bottles were each seeded with 5 ml. of inoculum and transferred to the growth chamber after 4 days' incubation at 25° C. The inoculum concentrations were 5, 20, 100, 200, and 300 thousand conidia per ml.; the yields, after a total of 7 days' incubation, were 2.5, 6.5, 6.0, 8.0, and 8.0 million spores, respectively, per gm. of substrate. Thus the optimum concentration of inoculum for this substrate seemed to be 200,000 conidia per ml. Further experiments confirmed this optimum and demonstrated that the higher concentrations or greater volumes of inoculum were not beneficial.

The optimum concentrations of inoculum given above for agar and whole-seed substrates were used throughout later experiments.

Substrates

Piricularia oryzae was studied on agar media in quadruplicate series of 8-ml. lots slanted uniformly in 18 × 150 mm. Pyrex test tubes. The spore yields were determined after 5 to 8 days' incubation at 24° C. Nineteen synthetic and 52 nonsynthetic agar media were tested.

The synthetic media included various sugars as carbon sources, inorganic and organic nitrogen sources, magnesium sulphate, potassium phosphate, agar, distilled water, and, in some cases, thiamin. There was no appreciable growth and no sporulation on any of these agars. The synthetic medium reported by Tochinai and Nakano (19) as supporting good growth and sporulation by *Piricularia oryzae* failed to support growth of our isolates. It was later shown (9) that both thiamin and biotin were required for the growth and sporulation of the isolates used in these studies.

The nonsynthetic agar media were made from many vegetables and cereals as well as several commercially prepared materials, *e.g.*, malt extract, yeast extract, beef extract, peptone, "Cerophyll," etc. All the nonsynthetic media supported growth of *Piricularia oryzae* to some degree. The requirements for sporulation were evidently more exacting. Yeast-extract, peptone, and barley-extract agars all gave good vegetative growth but no sporulation. Rice-polish, rice-bran, dried-green-rice-tissue, "Cerophyll," wheat, sorghum, oats-sorghum, and Bacto-malt agars gave good vegetative growth and abundant sporulation. It was evident that the luxuriant development of my-

celium was not always accompanied by the production of conidia, whereas the abundant production of conidia was always accompanied, or preceded, by considerable growth of mycelium. Rice-polish and rice-bran agars gave in general the highest spore yields, and the former was selected as the active ingredient in the stock culture medium. It was originally used at a 3 per cent concentration but later trials showed 2 per cent to be as good for sporulation, so this concentration was adopted. Representative data showing the quantity of sporulation by 5 isolates of *P. oryzae* on 3 nonsynthetic agars are presented in table 1. It was evident that the different media had a dissimilar influence on the quantity and stability of sporulation by the various isolates.

TABLE 1.—*Sporulation by 5 isolates of Piricularia oryzae subcultured at 5-day intervals on 3 different agars*[a]

Isolate[b]	2 per cent rice-polish agar					20 per cent sorghum agar					20 per cent oats-sorghum agar				
	Subculture					Subculture					Subculture				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1	7.7	10.6	6.7	5.6	9.6	1.7	6.5	4.7	4.8	5.1	1.3	3.3	3.5	4.7	10.3
17	4.2	6.7	9.7	3.2	1.9	0.8	3.4	3.0	2.1	3.7	2.5	3.1	2.0	1.5	1.6
27	10.1	11.2	6.1	3.1	1.2	3.7	2.9	1.6	0.7	0.3	3.4	2.3	0.4	0.0	0.1
42	25.7	7.1	6.8	11.0	18.5	0.9	3.8	5.5	8.4	9.9	4.5	2.7	4.5	2.2	8.1
54	14.8	7.5	4.7	1.3	0.4	0.7	1.9	1.8	2.3	3.9	1.9	1.5	1.5	0.7	1.1

[a] Data presented in millions of conidia per test tube culture. Each figure represents the average of 3 cultures.

[b] Isolate 1 was a subculture of the original strain. The other 4 isolates were obtained by host-passage of isolate 1.

Sporulation by *Piricularia oryzae* was studied on several whole-seed substrates in triplicate series of 100-gm. dry wt. samples plus the maximum amounts of water that would be imbibed during autoclaving. The seeded substrates were incubated at room temperature for 4 days in quart milk bottles and then transferred to the screen trays in photographic pans for an additional 4 days. Wheat, corn, oats, soybeans, cowpeas, and Hegari sorghum seeds were used separately. *P. oryzae* grew on all these substrates, with the most abundant development of mycelium on soybeans and cowpeas. The yields in millions of spores per gm. dry wt. of substrate were: wheat, 0.7; corn, 0.4; oats, 2.4; soybeans, 0.0; cowpeas, 0.0; and sorghum, 4.8. As with the agar substrates, abundant growth of mycelium did not ensure abundant production of conidia, since the two substrates which gave the most mycelial growth, namely, soybeans and cowpeas, yielded no conidia. It was evident that sorghum seed was the best of the group for the production of conidia. This substrate had the disadvantage however that the grains clumped together, presumably because of rupture during autoclaving. In an attempt to alleviate this difficulty, several materials of possible value as nutrient sources or as fillers, were mixed in various proportions with sorghum seed. Among these materials, rice hulls, peanut hulls, and the seeds

of oats, wheat, or corn aided in preventing the clumping of the sorghum; rice hulls and oats gave mixtures with the best physical qualities. The mixtures containing peanut hulls, rice hulls, and wheat all gave lower spore yields than sorghum alone, while mixtures containing certain proportions of oats and of corn gave higher yields than sorghum alone. The following are representative yields of the oats and corn mixtures in millions of conidia per gm. dry wt. of substrate: sorghum alone, 8.8; oats-sorghum at 6:5, 7.0; oats-sorghum at 1:1, 16.0; oats-sorghum at 3:5, 11.0; corn-sorghum at 6:5, 6.0; corn-sorghum at 1:1, 8.2; corn-sorghum at 3:5, 14.0. The 1:1 mixture of oats-sorghum was the best of the grain substrates from the standpoint of sporulation and also possessed the desired physical qualities. It was thus adopted as the substrate for production of conidia in quantity.

Aeration of Cultures

Piricularia oryzae produced conidia in abundance on suitable agar media in test tubes plugged with cotton. When cultured on agar in larger vessels, such as Roux flasks, relatively few or no conidia were produced even though other conditions were the same; likewise, few conidia were produced when the fungus was grown on 200-gm. dry wt. samples of a whole-seed substrate in 3-l. Fernbach flasks plugged with cotton, or in other semi-closed vessels. Brown (3) has reported ammonia to be a staling product produced by some fungi, and Mader (12) reported inhibition of mushroom fructification associated with a staling of the atmosphere about the cultures. After a few days' incubation, an ammonia-like odor could often be detected about the larger cultures of *P. oryzae* and wet litmus paper held in a culture vessel turned blue. These data indicated that there was a staling of the air about the cultures.[4] A series of experiments on the effect of forced aeration was begun.

The fungus was grown on sorghum seed in 3-l. Fernbach flasks equipped for the introduction of measured volumes of sterile, humidified air (Fig. 2). The rate of aeration was calculated in cc. of air/min./gm. of substrate. The effect on spore yields of rates up to 4.0 cc. are shown graphically in figure 4, A. It was evident that the rate of aeration had a pronounced effect upon sporulation and that the range of 0.0 (flask plugged with solid rubber stopper) to 4.0 cc./min./gm. did not go high enough to demonstrate the optimum. Sorghum was again used in one experiment and a 1:1 mixture of oats and sorghum was used in another in which higher rates of aeration were included. In both experiments, the sporulation curve levelled off at aeration rates of 3.3 to 4.0 cc./min./gm., and at about 5.0 cc./min./gm. or above sporulation was decreased. The latter may have resulted from partial drying of the substrate by the blast of air. The rate of 4.0 cc./min./gm. was concluded to be optimum for the grain substrates in Fernbach flasks.

[4] E. C. Tullis and A. L. Andersen (unpublished data) had previously obtained similar results with *Piricularia oryzae*.

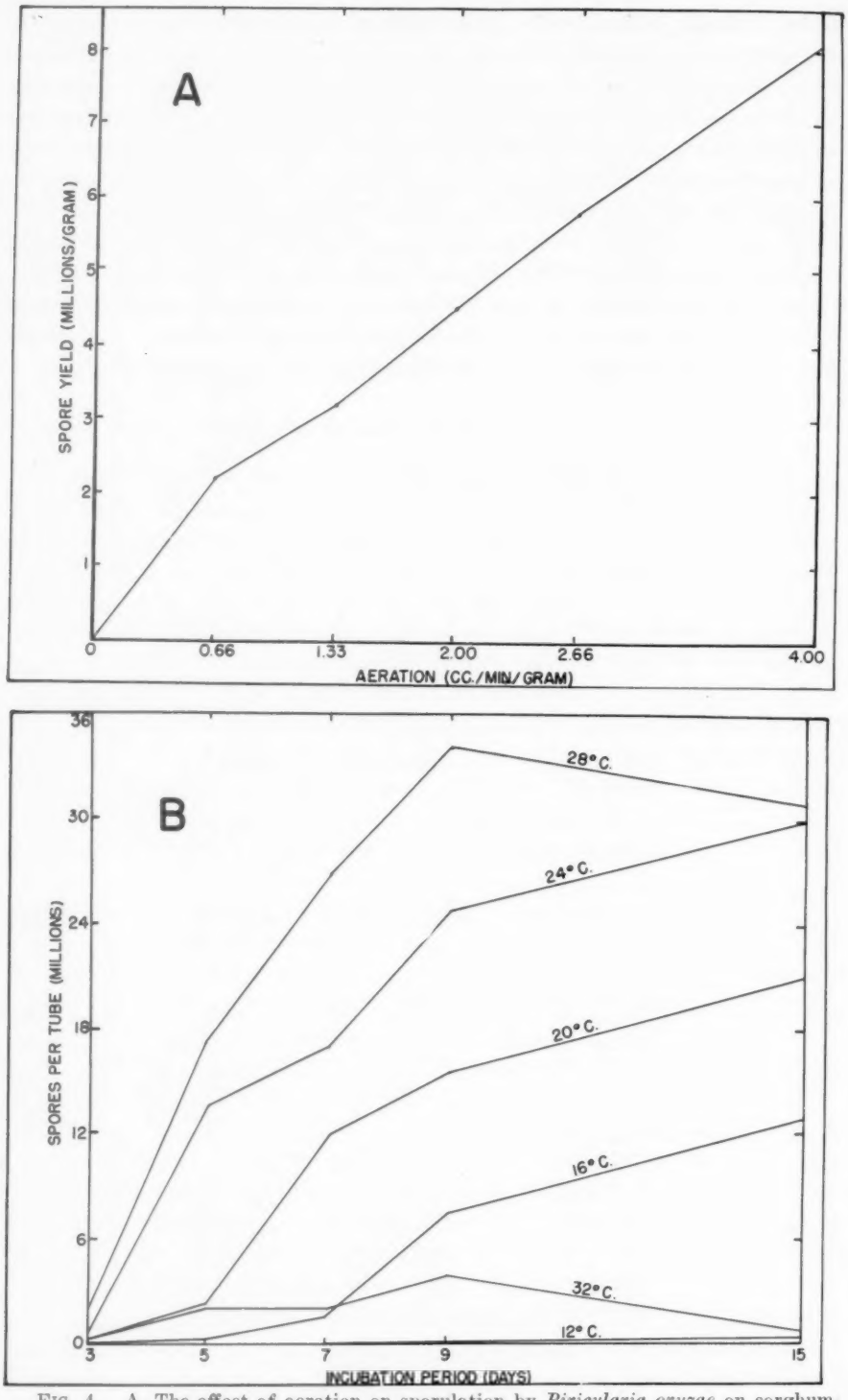

FIG. 4. A. The effect of aeration on sporulation by *Piricularia oryzae* on sorghum-seed substrate. B. The effects of temperature and time of incubation on sporulation by *P. oryzae* on rice-polish agar.

Moisture Content of Air

Hemmi and Imura (7) reported that conidia were produced by *Piricularia oryzae* on host-lesions in air maintained at 93 per cent or higher relative humidity. Andersen, Henry, and Tullis (1) concluded that high relative humidity was necessary for the production of conidia on the host. In the present studies, observations have shown that the air must be maintained at a high moisture content to obtain maximum sporulation on grain substrates. In practice, therefore, the air was humidified by bubbling it through sterile water. By governing the height of the water column and the size of the air bubbles it was possible to maintain the desired humidity without excessive condensation of moisture.

Moisture Content of Substrate

It was evident early in the course of these studies that high moisture content of the whole-seed substrates favored sporulation by *Piricularia oryzae*. It was desirable to incorporate the maximum amount of water that could be imbibed by the substrate without excessive bursting of the individual kernels. For the preferred oats-sorghum mixture, this amount was 1.15 times the weight of the dry grain. The two components of the substrate imbibed about equal amounts of the added water. If the water was added to the grain immediately prior to autoclaving, the result was excessive bursting of the sorghum kernels near the bottom of the layer of substrate with the upper part remaining too dry. This difficulty was alleviated by steeping the grain in hot water prior to autoclaving it. The desired amount of water was taken up by the oats-sorghum mixture in a 2-hour steep in an excess of water at 82° C., or in a 4-hour steep at 76° C. A less refined but faster method of steeping was to add the amount of water to the grain in individual culture vessels, heat the whole in an autoclave for about 15 min. at 107° C. and 3 to 5 lb. pressure, and remove and shake it while still hot. Sterilization of the substrate was accomplished by autoclaving in the usual manner. The latter method of steeping was used for most of the reported studies.

Time of Incubation

The effect of time of incubation upon sporulation by one isolate of *Piricularia oryzae* on rice-polish agar is shown in figure 4, B. Maximum sporulation at the optimum temperature (28° C.) was reached by the 9th day in this case. The sigmoid-type curve shows the most rapid sporulation to have taken place between 3 and 5 days after seeding, with a diminished rate from 5 to 9 days. Another isolate of *P. oryzae*, a comparatively poor sporulator, showed the same period of most rapid sporulation, but there was no further increase after 5 days. The time required for maximum sporulation may thus differ between isolates.

The time required for maximum sporulation on the oats-sorghum substrate varied with the way in which the cultures were handled. When they were incubated in milk bottles prior to being placed in aerated vessels, maxi-

mum sporulation was obtained after 4 days in the latter vessel, or a total incubation period of 7 to 8 days. Maximum sporulation was reached by the 6th day in cultures incubated in aerated Fernbach flasks.

Temperature of Incubation

The effect of temperature on sporulation by *Piricularia oryzae* was studied by incubating seeded tubes of rice-polish agar in cabinets held at 4° steps from 4° to 36° C. The amount of sporulation was determined at intervals up to 15 days (Fig. 4, B). Spores were produced most rapidly and in greatest quantity at 28° C. with the maximum yield of 34 million per tube. Spore production at 24° C. was not so rapid as at 28° C. but nearly equalled the latter after 15 days' incubation.

It is probable that the amount of sporulation at 16° and 20° C. would have reached the maximum set at 28° C. if the incubation period had been extended. There was no growth or sporulation at 4° or 8° C. The organism was not killed however, as some tubes removed from these temperatures after 9 days and incubated for an additional 6 days at 24° C. gave spore yields of approximately the same magnitude as the 6-day point on the 24° C. curve. There was good growth but little sporulation at 32° C. and slight growth but no sporulation at 36° C. The viability of the spores produced at 12° to 32° C. was unaffected but those produced at 32° C. developed short, knobby, abnormal germ tubes. Therefore, the optimum temperature for the production of conidia by *Piricularia oryzae* appeared to be 28° C. as reported by Tochinai and Shimamura (20). This temperature is also the optimum for mycelial growth as reported by Nisikado (14), and confirmed by Yoshii (23) and the present studies. Thus *P. oryzae* is similar to many other fungi in having the same optimum temperature for growth and sporulation (10, 21). At 28° C., however, sporulating isolates tended to give rise to white, asporogenous forms. Similar results with two species of *Magnusia* were reported by Sweet (18). To reduce this danger, the next lower temperature, 24° C., was used routinely for the incubation of all cultures.

Initial pH of Substrate

The effect of the initial pH of the substrate on sporulation by *Piricularia oryzae* was studied by incubating cultures on adjusted rice-polish agar at 24° C. and determining the number of spores produced per tube after 7 days. The various pH values were obtained by adding 0.1 N HCl or NaOH to the agar samples after autoclaving and in amounts determined from a titration curve prepared for rice-polish agar. The actual value of an aliquot sample in each case was determined with a Beckman pH meter. A pH range of 3.0 to 8.8 was used. Growth and sporulation occurred from 4.1 to 8.8 with maximum sporulation between 4.9 and 7.5. There was no growth on media more acid than pH 4.1. Nisikado (14) reported growth of *P. oryzae* throughout the pH range of 5.0 to 10.0. On the basis of these data, the two standard

substrates, rice-polish agar, pH 5.8 to 6.2, and 1:1 oats-sorghum substrate, pH 6.8, fell naturally within the optimum pH range for sporulation.

Frequent Subculturing

Twenty-five mass isolates and 25 single-spore isolates of *Piricularia oryzae*, all progeny of the original strain, were transferred weekly to fresh tubes of rice-polish agar for 9 weeks. At the end of selected incubation periods, the average spore yield for three tubes of each isolate was determined. Representative data are given in table 2. The results show a considerable amount of variation. The amount of sporulation increased in some isolates, decreased in most, and fluctuated in all during the 9-week period. The mass isolates and single-spore isolates were alike in this respect.

TABLE 2.—*Sporulation by representative isolates of Piricularia oryzae subcultured weekly for 9 weeks on rice-polish agar*[a]

Isolate	Subculture					
	1	2	3	5	7	9
42	10.0	16.0	8.3	8.0	5.0	3.6
42–A[b]	8.0	11.3	9.7	5.8	5.8	9.0
42–B[b]	2.3	1.1	1.4	2.7	2.9	1.6
42–C[b]	7.3	14.6	11.6	8.0	7.0	7.2
42–D[b]	2.5	1.4	2.7	4.3	2.9	1.2
42–1[c]	7.9	4.6	8.8	5.5	10.5	14.0
42–2[c]	11.4	10.5	11.6	2.3	2.4	0.7
42–3[c]	8.7	11.9	9.5	6.1	4.3	5.6
42–3–1[d]	.....	.....	10.4	6.3	1.8	0.8
42–3–2[d]	.....	.....	9.7	7.2	2.4	1.3
42–3–3[d]	.....	.....	12.8	8.6	2.6	5.3

[a] Data in millions of conidia per test-tube culture. Each figure represents the average of 3 cultures.
[b] Reisolates obtained by host-passage of isolate 42.
[c] Single-spore isolates from isolate 42.
[d] Single-spore isolates from isolate 42–3.

Other high-sporulating isolates kept as stock cultures were subcultured frequently on rice-polish agar for periods up to 15 months. The amount of sporulation fluctuated considerably between subcultures, but the general tendency over a long period was always toward decreased sporulation. Greene (6) reported a similar tendency in high-sporulating cultures of *Aspergillus fischeri*.

Storage of Isolates at Low Temperatures

Transfers of all isolates of *Piricularia oryzae* used in these and allied research studies were stored in tubes of rice-polish agar at 6° to 8° C. Some cultures were untreated and others were covered with a layer of sterile mineral oil (16). Cultures stored with or without oil were viable for at least 13 months. Fresh transfers of most cultures that were stored under oil for 13 months gave higher sporulation than fresh transfers of like cultures that were stored without oil. Cultures better retained their ability to

sporulate when stored at 6° to 8° C., with or without oil, than when they were frequently subcultured at 24° C. over a like period of time.

Host Passage of Isolates

About 100 different isolates of *Piricularia oryzae* were obtained by plant passage of the original strain. The amount of sporulation by subcultures of many of these on rice-polish agar was studied. The variation in the amount of sporulation between and within these isolates was great (See tables 1 and 2). Within a group of isolates obtained from lesions on identical plants that had been inoculated simultaneously with the same lot of inoculum, there appeared some that sporulated more and some that sporulated less than the isolate used as inoculum. The magnitude of the difference varied between groups. Repeated host passage of a particular isolate did not seem to lessen the variation in sporulation between or within its reisolates. In spite of the tremendous amount of variation, it has been possible to retain the high-sporulating characteristic of isolates of *P. oryzae* for 3 years by means of host passage.

RELATION OF SPORULATION TO MACROSCOPIC CHARACTERS

The observation of macroscopic characters accompanied the study of sporulation by *Piricularia oryzae*. A so-called normal colony of *P. oryzae* was olive-gray with aerial hyphae to a height of 1 to 3 mm. Most of the high-sporulating isolates were of this type, but few were darker with more appressed aerial growth. The non-sporulating isolates were much lighter to nearly white, with a 3 to 10 mm. fluffy layer of aerial hyphae. There were all gradations between the two extremes. The original strain was of the normal, sporulating type, and gave rise to all the other types encountered in this study. The non-sporulating types arose in different ways, such as incubation at 28° to 32° C., frequent transfer on rice-polish agar, growth on peptone, yeast-extract, and barley-extract agars, and on seeds of soybeans and cowpeas. There was also the appearance, in some cases, of a non-sporulating patch sector not evidently associated with any of these environmental conditions. Such sectors may have been mutants. Stevens (17) and Christensen (4), working with *Helminthosporium sativum* P. K. and B., reported that pale, poor-sporulating mutants occurred most frequently. Figure 1, B, illustrates the macroscopic appearance of some *P. oryzae* isolates on oats-sorghum substrate.

DISCUSSION

Sporulation by *Piricularia oryzae* is a process influenced by several factors. Given an isolate inherently capable of sporulating, the first requisite is a certain amount of vegetative mycelium. Those factors basically necessary for the production of the vegetative thallus might therefore be listed as necessary for sporulation. Some of these factors, such as pH of the substrate and temperature of incubation, seem to affect sporulation largely as

a result of their effects on vegetative growth. The optimum state of such factors appears to be about the same for the growth and sporulation processes. Other required factors, such as the specific substrate, the moisture content of the substrate and the adjacent atmosphere, and aeration of the culture, may meet the requirements for vegetative growth but be unsuitable for sporulation. Forced aeration, for example, did not seem to be required for vegetative growth of *P. oryzae* under the experimental conditions, but was required for sporulation in culture vessels larger than the ordinary test tube. Certain substrates, such as whole-seeds of soybeans or cowpeas, supported luxuriant development of mycelium but no sporulation, thus indicating that nutrients and growth factors in quality and quantity favorable for vegetative development may not be favorable for spore development. Hence the requirements for sporulation by *P. oryzae* are more exacting, and possibly more numerous, than the requirements for growth.

SUMMARY

The process of spore formation by *Piricularia oryzae* in artificial culture was found to be similar to that on the host.

High-sporulating isolates were usually olive-gray with a 1 to 3 mm. layer of aerial hyphae. The relatively nonsporulating isolates were a lighter color to nearly white, with a 3 to 10 mm. layer of aerial hyphae.

High-sporulating isolates were best retained in that condition by storage under mineral oil at 6° to 8° C.

Of the several agar and whole-seed substrates suitable for spore production, the best were 2 per cent rice-polish agar and a 1:1 mixture of oats-sorghum, respectively. Spore yields were increased with increased concentrations of inoculum up to an optimum range.

Moisture contents of near saturation for the whole-grain substrates and the adjacent atmosphere favored abundant sporulation.

Forced aeration was necessary for sporulation in culture vessels larger than the ordinary test tube. A rate of about 4 cc. of air/min./gm. of oats-sorghum substrate was optimum.

The length of the incubation period required for highest sporulation varied with the temperature, the culture vessel, and, to some extent, with the isolate.

The effects of incubation temperatures and pH of the substrate on sporulation appeared to be a result of their effects on growth. The optimum state of these factors, 28° C. and pH 4.9 to 7.5, appeared to be about the same for growth and sporulation.

Isolates of the pathogen, obtained by host-passage of the original strain, followed in some cases by single-sporing, varied considerably in the quantity of spores produced in a given environment.

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AN ELECTRON MICROSCOPE STUDY OF TOBACCO MOSAIC VIRUS EXTRACTED FROM PULP AND JUICE AFTER VARIOUS PERIODS OF INFECTION

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The mode of virus multiplication or increase is among the most interesting problems in pathology. An earlier attempt (2) to gain evidence on this process by studying the length of virus particles in juice from leaves infected for various periods was described in 1946. Bawden (1) has reported that tobacco mosaic virus obtained from finely macerated leaf pulp has shorter particles than does virus obtained from juice. In the work described here we have tried to get the virus from finely macerated pulp as well as from the juice and have avoided the use of ultracentrifugation, chemical treatments, or filtration which may cause aggregation or loss of virus. It was hoped that an electron microscope study of combined pulp and juice virus obtained at intervals after infection might provide evidence regarding the changes that occur in virus particles during multiplication or increase.

METHODS

Unfrozen tobacco leaves which had been infected for various periods of time were finely ground in a hand operated ground glass homogenizer to try to free the virus in both pulp and juice. One gram fresh weight of leaf blade was used for each sample. Five cc. of distilled water were added during the grinding to produce a liquid suspension of the pulp and juice. This mixture had the pH of plant juice, around 5.6. The mixture was then centrifuged at about 2500 r.p.m. for 1 hour to remove the coarser material. The supernatant was diluted with distilled water and placed on celloidin membranes for electron microscopy.

RESULTS

As in earlier work (2) it was found that the lengths of tobacco mosaic particles usually fall into 3 fairly distinct groups. The length distribution curves usually show a group of the shortest particles having lengths under 225 mμ, a group of medium length particles having lengths between 225 and 463 mμ, with a rather high peak around 300 mμ, and a third group composed of the longest particles having lengths greater than 463 mμ. In the length distribution curves the middle group is usually separated from the other groups by distinct valleys indicating that the particles in the shortest and longest groups are not simply variants in a normal distribution about the middle group.

The proportion of the particles in each group after various periods of infection are shown in table 1. The results indicate that the proportion of

the particles in the shortest group becomes greater as multiplication proceeds between the fourth and sixteenth day after inoculation of the leaf. The proportion in the middle group did not show any distinct change with increasing age of infection.

TABLE 1.—*Proportion of virus particles in each size group when extracted at pH 5.6 at intervals after infection*

Experiment no.	Age of infection in days	No. of particles measured	Percentage shorter than 225 mμ	Percentage between 225 and 463 mμ long	Percentage longer than 463 mμ
31	4	124	11	43	44
34	4	156	15	43	42
34	6	150	10	14	74
31	6	79	23	40	37
33	7	529	20	69	9
34	9	290	31	54	14
33	12	394	35	60	6
34	12	330	32	37	31
31	13	365	40	37	23
34	16	1022	65	30	5
31	20	234	45	38	17
33	20	709	44	51	5
34	20	722	60	32	7

The proportion in the long group showed some tendency to decrease after the first six days of infection but the results showed considerable variation.

DISCUSSION

Perhaps the most interesting result obtained is the apparent increase in the proportion of particles in the short group during the process of virus multiplication. The combined results of Stanley (5), Spencer (4), and Rawlins, Roberts, and Utech (2) have given ample evidence to prove that there is a great increase in the number of middle group particles per cell during virus multiplication. Since the proportion of short particles apparently increases at the same time it may be concluded that there is also a great increase in the number of short group particles per cell during virus multiplication. Such a conclusion would be justified as long as there is no decrease in the proportion of short group particles during virus multiplication. The grinding of purified middle group particles from juice with the ground glass homogenizer has failed to change the length of the particles or to decrease their infectivity. The same result was obtained when the middle group virus was mixed with healthy pulp before grinding. These results apparently indicate that the increase in the proportion in the short group did not result from the action of the homogenizer on middle group particles. Since the results of Stanley (5) and Spencer (4) indicate that leaves infected for 20 days have from 3 to 7 times more middle group particles per cell than leaves infected for 7 days it is obvious that changes in the proportion

of particles in the 3 groups during virus multiplication may be due partly or entirely to different rates of increase of the 3 groups rather than to one group being derived from another. The results shown in table 1 therefore cannot be considered as evidence for or against the short group being derived by the breaking up of particles in one or both of the longer groups. Earlier work with juice virus (2) failed to show any distinct increase in the proportion of short group particles during virus multiplication. The increase shown in table 1 is therefore probably due to an increase in the proportion of short particles from pulp virus which was included in our samples along with the juice virus.

There is considerable uncertainty regarding the infectivity of the short group particles from pulp. Sigurgeirsson and Stanley (3) have given evidence that an unsedimentable fraction from infective plant juice, consisting mainly of short particles, shows little infectivity. They did not study the infectivity of the short particles from pulp. Some of our unpublished results indicate that about 30 per cent of the local lesions produced by virus extracted from pulp alone are produced by short group particles and that the other 70 per cent are produced by middle group particles. Other of our experiments indicate that all of the infectivity in the pulp virus is due to middle group particles. Accordingly more evidence must be obtained before conclusions can be reached as to whether all of the short particles from pulp are noninfective.

None of the results shown here or in the literature appears to give a satisfactory explanation of the origin of the short group particles or of the significance of this group in virus multiplication.

The mode of formation of the middle group also remains undetermined. All of our attempts to produce a distinct middle group by artificial aggregation of short group particles have been unsuccessful. This result apparently constitutes evidence against the middle group particles having been formed by the aggregation of short group particles.

Long group particles, on the other hand, may be readily produced at will by ultracentrifuging a mixture of short group and middle group particles in distilled water. Extraction of combined pulp and juice virus in phosphate buffer at pH 7 instead of distilled water prevents the formation of an appreciable number of long group particles. All of this evidence apparently indicates that the long group results by aggregation of middle group and short group particles when the pH is around 5.6. This tendency is particularly pronounced in juice from leaves which have been infected for 6 days or less.

SUMMARY

When tobacco mosaic virus is extracted from finely macerated pulp and juice at the pH of plant juice the proportion of short particles increases between the fourth and sixteenth days after infection. The concentration of short particles per cell, like that of middle group particles per cell, in-

creases during this period. The short particles did not result from fracture of middle group particles during maceration.

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THE SPECIES VALUE OF PATHOGENICITY IN THE GENUS XANTHOMONAS

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Elliott (5) has reviewed, from an historical point of view, the important changes in the classification and nomenclature of the bacterial plant pathogens. The changes brought about have been concerned primarily with disposition of the species of the outmoded genus *Phytomonas* into new or older genera emended to include the plant pathogens. For the moment, generic characters have been stabilized and White's (18) simple key for the working pathologist should be revised.

Keys to differentiate species within the genera are not too adequate. Burkholder (2) points out two contributing factors: (i) many of the species can be separated only on their pathogenicity to certain plants or groups of plants, *i.e.*, physiological tests are as yet inadequate and (ii) cross inoculations with the hosts of organisms of similar appearance and apparent physiological identity have not always been sufficiently complete to eliminate synonymy. With respect to these factors Burkholder urges a continued search for physiological tests of diagnostic value and points out the general areas where cross inoculation investigations might provide profitable information (2, p. 134). Stapp (12, p. 409) advocates "comprehensive comparative studies of the species . . . involving serological tests in addition to the usual study of morphological, cultural, and physiological characters."

Within the genus *Xanthomonas* of Dowson (4) numerous cross inoculation studies have been conducted. Those data, however, are scattered throughout the literature, but a glance through Elliott's *Manual of Bacterial Plant Pathogens* shows numerous species of plants susceptible to various organisms, which has been the result of cross inoculation work. It is a misfortune, in this instance, that she has not listed those plants that were not susceptible when tested. Furthermore, in the *translucens* group, Jones, Johnson, and Reddy (10), Hagborg (9), and Wallin (16) have made numerous cross inoculations among the cereals and have shown that phytogenic strains of the bacteria exist, not unlike those found in the rust fungi. In these inoculations recent isolates were used so that loss of pathogenicity of the various cultures could not account for the differences in the strains.

[1] The author gratefully acknowledges the kindly and constructive criticisms of Dr. W. H. Burkholder throughout the investigation and during the preparation of the manuscript.

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Progress has been reported too in the field of physiological tests. The contributions of Starr and Burkholder (14) and of Starr (13) point clearly to the fact that some species at present incorporated in *Xanthomonas* (*X. rubrilineans*) show few cultural characteristics relative to the genus as a whole, whereas others have exacting nutritional requirements, which may not be satisfied by synthetic basal media.

A recent paper by Elrod and Braun (6) presents a study of the genus by cross agglutination tests. These authors studied 36 species and subspecies of *Xanthomonas* and on the basis of their observations on cross agglutination reactions divide the species into five serological groupings as follows:

I. The *campestris* group comprising *X. campestris* and *X. barbareae.*
II. The *vascularum* group comprising *X. vasculorum, X. vesicatoria* and variety *raphani, X. hederae, X. papavericola, X. incanae, X. campestris* var. *amoraceae,* and *X. taraxaci.*
III. The *phaseoli* group comprising *X. phaseoli* and its variety *fuscans, X. geranii, X. pelargonii,* and *X. malvacearum.*
IV. The *translucens* group comprising *X. translucens* and its races, *X. begoniae, X. cucurbitae, X. juglandis, X. carotae,* and *X. maculafolium-gardeniae.*
V. The *pruni* group comprising *X. pruni, X. corylina, X. phaseoli* var. *sojensis,* and *X. lespidizae.*

Six of the species or subspecies could not be placed in any group. The authors regard three of these (*X. hyacinthi, X. rubrilineans,* and *X. manihotis*) as doubtful xanthomonads. The remaining three *X. gummisudans, X. vignicola,* and *X. holciola*) did not show any serological affinity with the other organisms under study.

As a result of their researches Elrod and Braun (6) suggest a reduction in the number of species within the genus.

During the period 1934–36 the writer (17) worked on the taxonomy of the "campestris" group of the genus *Phytomonas* as defined by Burkholder (1). This is the present genus *Xanthomonas.* Cross inoculation studies among 16 taxonomically distinct hosts was a phase of the investigation. Inasmuch as 17 members inclusive of 14 species and 3 subspecies and representing 37 isolates (Table 1) are common to the investigations of both Elrod and Braun and the writer, the results of the writer have a distinct bearing on the problem; and although the results have been referred to in the literature (2, p. 134), it seems advisable to present them in greater detail in order to compare cross agglutination reactions with pathogenicity data for the purpose of determining species within the genus.

MATERIALS AND METHODS

Isolates were contributed by many people. Five were isolated by the author. For convenience, these are grouped alphabetically by species; the

TABLE 1.—*Results[a] of cross inoculation experiments with isolates of species of Xanthomonas.*

Species of *Xanthomonas*	Serological Group[b]	Isolate[c]	*Brassica oleracea* var. *capitata*	*Corylus avellana*	*Cucurbita maxima*	*Geranium sanguineum*	*Hedera helix*	*Juglans regia*	*Gossypium hirsutum*	*Papaver rhoeas*	*Phaseolus vulgaris*	*Glycine max*	*Prunus persica*	*Saccharum officinarum*	*Hordeum vulgare*	*Triticum aestivum*	*Lycopersicon esculentum*	*Matthiola incana*
1. *campestris*	I	1	+	0	–	0	–	0	–	–	–	–	–	–	–	–	–	–
		27	+	0	–	0	–	0	–	–	–	–	–	–	–	–	–	–
		*37	–	0	–	0	–	0	–	–	–	–	–	–	–	–	–	–
		57	+	0	–	0	–	0	–	–	–	–	–	–	–	–	–	–
2. *corylina*	II	78	–	+	–	0	–	–	–	–	–	–	–	0	–	–	–	0
3. *cucurbitae*	IV	*41	–	0	+	0	–	0	–	–	–	–	–	–	–	–	–	0
		45	–	0	+	0	–	0	–	–	–	–	–	–	–	–	–	0
		46	–	0	+	0	–	0	–	–	–	–	–	–	–	–	–	0
4. *geranii*	III	80	–	0	–	?	–	0	–	–	–	–	–	0	–	–	–	0
		81	–	0	–	+	–	0	–	–	–	–	–	0	–	–	–	0
5. *hederae*	II	16	–	0	–	0	+	0	–	–	–	–	–	–	–	–	–	0
6. *juglandis*	IV	77	–	–	–	0	–	+	–	–	–	–	–	–	–	–	–	0
		79	–	–	–	0	–	+	–	–	–	–	–	0	–	–	–	0
		82	–	–	–	0	–	+	–	–	–	–	–	0	–	–	–	0
7. *malvacearum*	III	64	–	0	–	0	–	0	+	–	–	–	–	–	–	–	–	0
8. *papavericola*	II	47	–	0	–	0	–	0	–	+	–	–	–	–	–	–	–	0
		48	–	0	–	0	–	0	–	+	–	–	–	–	–	–	–	0
9. *phaseoli*	II	5	–	0	–	0	–	0	–	–	+	–	–	–	–	–	–	0
		6	–	0	–	0	–	0	–	–	+	–	–	–	–	–	–	0
		7a	–	0	–	0	–	0	–	–	+	–	–	–	–	–	–	0
10. *phaseoli* var. *fuscans*	II	8	–	0	–	0	–	0	–	–	+	–	–	–	–	–	–	0
		9	–	0	–	0	–	0	–	–	+	–	–	–	–	–	–	0
11. *phaseoli* var. *sojensis*	V	11	–	0	–	0	–	0	–	–	±	+	–	–	–	–	–	0
12. *pruni*	V	21	–	0	–	0	–	0	–	–	–	–	+	–	–	–	–	0
		22	–	0	–	0	–	0	–	–	–	–	+	–	–	–	–	0
		24	–	0	–	0	–	0	–	–	–	–	+	–	–	–	–	0
		59	–	0	–	0	–	0	–	–	–	–	+?	–	–	–	–	0
13. *rubrilineans*	VI	63	–	0	–	0	–	0	–	–	–	–	–	+	–	–	–	0
14. *translucens*	IV	61	–	0	–	0	–	0	–	–	–	–	–	–	+	–	–	0
15. *translucens* var. *undulosum*	IV	62	–	0	–	0	–	0	–	–	–	–	–	–	–	?	–	0
		83	–	0	–	0	–	0	–	–	–	–	–	0	–	+	–	0
		84	–	0	–	0	–	0	–	–	–	–	–	0	–	+	–	0
16. *vascularum*	II	12	–	0	–	0	–	0	–	–	–	–	–	+	–	–	–	0
		49	–	0	–	0	–	0	–	–	–	–	–	+	–	–	–	0
17. *vesicatoria*	II	13	–	0	–	0	–	0	–	–	–	–	–	–	–	–	+	0
		38	–	0	–	0	–	0	–	–	–	–	–	–	–	–	+	0
		*39	–	0	–	0	–	0	–	–	–	–	–	–	–	–	+	0

[a] + = positive
– = negative
± = both positive and negative results
0 = no data
? = doubtful at time of writing

[b] I–VI = the serological group number of Elrod and Braun (6).

[c] * = albino strain

serological grouping of Elrod and Braun (6) is indicated by roman numerals.

(i) *Xanthomonas campestris* (Pammel) Dowson. Group I.

1. from cauliflower, Aug., 1929. Dr. W. H. Burkholder, Ithaca, New York.
27. from broccoli, Aug., 1932. Wernham, Ithaca, New York.
37. an albino strain received from Miss Mary K. Bryan, Washington, D. C. Sept., 1932.
57. from cabbage, Sept., 1932. Wernham, Ithaca, New York.

(ii) *X. corylina.* Miller *et al.* Group II.

78. from English filbert. Dr. P. W. Miller, Corvallis, Oregon. Received May 15, 1935.

(iii) *X. cucurbitae* (Bryan) Dowson. Group IV.

41. an albino strain received from Miss Mary K. Bryan. Sept., 1932.
45. from cucumber, April, 1933. Miss Mary K. Bryan.
46. from cucumber, April, 1933. Miss Mary K. Bryan.

(iv) *X. geranii* (Burkholder) Dowson. Group III.

80. from *Geranium sanguineum,* June 4, 1935. Dr. W. H. Burkholder.
81. ditto.

(v) *X. hederae* (Arnaud) Dowson. Group II.

16. from *Hedera helix* collected at Rennes, France, Feb., 1932. Dr. W. H. Burkholder.

(vi) *X. juglandis* (Pierce) Dowson. Group IV.

77. from English walnut. Dr. P. W. Miller. Received May 15, 1935.
79. from English walnut. 1933. Dr. P. A. Ark, Berkeley, California. Received May, 1935.
82. from English walnut, collected at Ukiah, California by Mr. C. E. Scott. May 24, 1935. Wernham.

(vii) *X. malvacearum* (E.F.S.) Dowson. Group III.

64. from cotton, 1931. Miss Mary K. Bryan. Received Nov., 1933.

(viii) *X. papavericola* (Bryan and McWhorter) Dowson. Group II.

47. from Shirley poppy, 1932. Miss Mary K. Bryan. Received April, 1933.
48. from Oriental poppy, 1931. Miss Mary K. Bryan. Received April, 1933.

(ix) *X. phaseoli* (E.F.S.) Dowson. Group II.

5. from bean pods collected in Switzerland, Oct., 1927. Dr. W. H. Burkholder.
6. from bean seed collected at Clifton Springs, N. Y., 1923. Dr. W. H. Burkholder. April, 1930.
7a. a reisolate of a culture obtained from bean seed by Miss H. Nussle, Ithaca, N. Y. Wernham. (Subsequent to inoculation the original culture was lost.)

(x) *X. phaseoli* var. *fuscans* (Burkholder) Starr and Burkholder. Group II.

8. from bean pods collected in Switzerland, Dec., 1924. Dr. W. H. Burkholder.
9. ditto. Oct., 1927.

(xi) *X. phaseoli* var. *sojensis* (Hedges) Starr and Burkholder. Group V.

11. from soybeans. Miss Florence Hedges, Washington, D. C. Sent to Dr. W. H. Burkholder, March, 1930.

(xii) *X. pruni* (E.F.S.) Dowson. Group V.

21. from prune, Aug. 16, 1932. Dr. W. H. Burkholder.
22. ditto.
23. isolated by Dr. H. H. Thornberry, Boyce Thompson Institute, Yonkers, N. Y. Received Aug., 1932.
59. from wild cherry collected at Georgetown, N. Y., Aug. 28, 1933. Wernham.

(xiii) *X. rubrilineans* (Lee *et al.*) Starr and Burkholder. Group VI.

63. received Nov., 1933, from Miss Florence Hedges through the courtesy of Miss Charlotte Elliott.

(xiv) *X. translucens* (Jones *et al.*) Dowson. Group IV.

61. from barley, September., 1933. Dr. C. S. Reddy, Ames, Iowa.

(xv) *X. translucens* var. *undulosum* (S. J. and R.) Hagborg. Group IV.

62. from wheat, Sept., 1933. Dr. C. S. Reddy.
83. received from Dr. R. A. Bamberg, University Farm, St. Paul, Minnesota, March 27, 1936.
84. ditto.

(xvi) *X. vascularum* (Cobb) Dowson.

12. isolated in 1923 at Bureau of Plant Industry. Received by Dr. W. H. Burkholder, March, 1930.

49. from sugar cane. Dr. Mel. T. Cooke, Rio Piedras, Porto Rico. Received May, 1933.

(xvii) *X. vesicatoria* (Doidge) Dowson. Group II.

13. from tomato fruits, Aug., 1929. Dr. W. H. Burkholder.
38. received from Miss Mary K. Bryan, Sept., 1932.
39. an albino strain received from Miss Mary K. Bryan, Sept., 1932.

All bacteria used were tested for pathogenicity on their original suscept, and those that were non-virulent were discarded.

Most of the plants used in the inoculation experiments are the hosts on which the pathogens were originally described. Of these plants the following were grown from seeds: cabbage, cotton, Shirley poppy, tomato, soybean, common bean, squash, barley, and wheat. Potted plants of English ivy and stocks were used. Cuttings of sugar cane were received from Louisiana. Six plants each of English walnut and English filbert were purchased from a commercial firm. June buds of Elberta peaches were furnished by a nursery. Only one plant of *Geranium sanguineum* was available.

Three methods were used in inoculating plants. Transfers from stock cultures were made to Difco nutrient agar slants and incubated for 48 hours at 27° C. The bacteria were (a) smeared over the leaf or applied in water suspension, and pricked through by means of a sheaf of sterile insect needles or (b) scraped off the slant on the tip of a sterile scalpel and introduced into the vascular system on the scalpel point or (c) atomized or brushed onto leaves by means of sterile atomizers or camel's hair brushes.

After inoculation species of small plants were placed in an incubation chamber of 85–95 per cent relative humidity for 48 hours. Wherever possible two series of plants were used, each series at a different time in the investigation. Each series allowed at least one plant; individual leaves made possible many single inoculations. The material of sugar cane, walnuts, and filberts was too scarce for more than pathogenicity tests. Cultures added to the study after scarce material was discarded were not tested. Of all the cross inoculations made, only the results on sugar cane are subject to misinterpretation. With this species symptoms were confusing.

Infection was considered positive only when the bacteria were able to establish themselves and cause a progressive disease. Thus injury due to needle pricks plus toxic material of the inoculum was considered negative.

CROSS INOCULATIONS

A study of the cross inoculation results shows this group of pathogens to be remarkably specific in their host reactions (Table 1). This is in marked contrast to the results reported by Clara (3) in his study of the green fluorescent plant pathogens. Some yellow isolates which were re-

ceived as authentic cultures proved to be non-pathogenic in spite of repeated attempts to rejuvenate them, and they are not listed here. Of the three albino strains included in the study, two were pathogenic, showing that a color mutation is not necessarily accompanied by loss of virulence.

Pathogenicity as a species concept is definitely of value in the species studied. With the single exception of the subspecies *phaseoli fuscans* which can be distinguished readily from *phaseoli* by the soluble brown pigment it imparts to the agar, there is no possibility of error in using the host as a species determiner.

Both *Xanthomonas vascularum* and *X. rubrilineans* infect sugar cane but produce different symptoms. Furthermore, while the former is a true species of *Xanthomonas,* the latter shows few cultural characteristics of the genus according to Starr and Burkholder (14). Serologically Elrod and Braun also found no relationships.

The five serological groups of Elrod and Braun show no correlation with the pathogenicity of the various species investigated in this article. Furthermore, the species of this genus that hydrolyse or do not hydrolyse starch, the few species that are not lipolytic (14), the one species that breaks down tyrosine into a melanin (*X. phaseoli* var. *fuscans*) and the "fastidious" species of Starr (13) while they help in defining the phytogenic species do not correlate with the serological grouping. Although it is of interest to know the serological relationships in the genus, the plant pathologist will insist on a nomenclature to differentiate the phytopathogenic species.

DISCUSSION

In order to place the data presented above in proper perspective it appears to be necessary to present the cogent reasons for the serological study of Elrod and Braun. These authors (6, p. 509) state:

"Since establishment of the genus, the more than 40 members have assumed a more rational place in bacteriological taxonomy but are still little understood as a group. It is unfortunate that these organisms . . . , have received little study. We have scant knowledge of the relationships between the various species and subspecies. Inasmuch as it is the practice of the phytopathologist in general to form a new species if a bacterial plant pathogen of a recognized genus is isolated from a new host, it is apparent that many species could have been duplicated. This practice of basing species differentiation almost solely on host of isolation (with the host range almost always inadequately investigated) has resulted in a seeming increase in the size of the genus, giving rise to the belief among certain workers that all the members may not represent true species.

As an approach to this problem we have undertaken an extensive serological survey of the group to determine whether the genus could be logically classified in this fashion and to ascertain whether antigenic characteristics could be correlated with host range."

Examination of the references cited by these authors reveals that the

literature (4, 8, 9, 13, 14, 16) had been covered with the possible exception of Burkholder's article of 1939 (2). It is difficult indeed to interpret the polemical approach and its reiteration throughout the discussion, had the authors been aware of this paper. Nevertheless the data are established and it is necessary to evaluate them in the light of cross inoculation results presented above and that previously published by others.

The serological relationships do not correlate with the host range as determined by the author, nor do the results of Wallin (16) coincide with the groupings of the *translucens* isolates in which we find *X. translucens* f. sp. *hordei* in both the "cucurbitae" and the "cerealis" subgroups. These data are also at variance with the serological findings of Hagborg (9). A more recent paper (7) by Elrod and Braun emphasizes these discrepancies. In this publication (7, p. 356) the authors state: "It is felt that the antigenic properties of an organism vary independently with the ability to infect given host species."

These findings and statements are in general agreement with those of Stevens (15) who carried on similar investigations with the legume nodule-forming bacteria. Seven species of these bacteria, based on host plant infection, were studied. Agglutination differentiated the 55 cultures into 18 serological groups. Stevens' results indicated "the impossibility of identifying an organism by means of the agglutination test."

The writer believes his data, together with those of Hagborg (8), Wallin (16), and earlier workers (10) are sufficiently ample to show clearly that serological studies, regardless of their potential usefulness, are subordinate in value to pathogenicity as species determiners in the genus *Xanthomonas* at the present level of our information.

The writer believes, in addition, that any technique of species identification that is beyond the immediate faculties of the research worker primarily concerned with the species, fails as an implement of usage in any system of classification.

SUMMARY

1. Cross inoculation studies with 17 members of the genus *Xanthomonas* on 16 taxonomically distinct hosts reveal pathogenicity to be a remarkably specific characteristic of these species.

2. There is an almost complete lack of correlation between pathogenicity and the serological grouping of the species as reported by Elrod and Braun (6).

3. The data indicate that, within the genus, pathogenicity is of primary consideration as a species determiner, in the light of our present knowledge of the group.

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A TOXIC METABOLIC PRODUCT OF FUSARIUM OXYSPORUM VAR. NICOTIANAE IN RELATION TO A WILTING OF TOBACCO PLANTS[1]

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Among the theories attempting to explain the wilting of plants infected with vascular parasites of the genus *Fusarium*, the toxin theory has been supported by most investigations within recent years and has gained most wide acceptance. This theory is based primarily upon observations that these fungi, when grown upon artificial media, produce toxins capable of wilting healthy plants. Organisms for which the production of wilt-inducing toxins have been demonstrated include *Fusarium cubense*, causing the Panama disease of banana (2, 16), *F. solani* (5, 21), *F. oxysporum* from potato (1, 9, 13, 28), the cotton wilt organism, *F. vasinfectum* (18, 20, 21, 24, 25), the pea wilt organism, *F. orthoceras* var. *pisi* (17), *F. lini*, causing flax wilt (12, 19), and the tomato wilt organism, *F. lycopersici* (or *F. bulbigenum* var. *lycopersici*) (3, 6–8, 10, 11, 14, 19, 22, 23, 26, 27). The literature on the wilting problem has been recently summarized by Gottlieb (11) and hence will not be considered in detail.

The toxin theory has been strengthened by the findings of Wellman (26), who reported differences in the toxicity of culture filtrates of mild and virulent strains of *Fusarium lycopersici*, increased toxin production being associated with greater virulence. In *F. lycopersici*, Gottlieb (10) has provided direct evidence in support of the toxin theory by the demonstration of toxins within the tracheal systems of wilted plants, this constituting the only instance for any Fusarium disease in which a toxin has been recovered from an infected host plant.

Efforts to identify the chemical compounds concerned in wilting have been successful in relatively few cases. The toxic metabolic product of various Fusaria has been identified as an aldehyde (16), nitrite (24), an amine (12, 18, 25), ammonia (4, 19), and an alcohol (19). Recent studies by a group of Swiss investigators have resulted in the isolation and characterization of a polypeptide-like toxin from filtrates of *Fusarium lycopersici* (3, 22, 23). The culture filtrate was treated with $Ba(OH)_2$ to remove sulphate and phosphate ions, and evaporated to dryness *in vacuo*. The residue was then dissolved in water and the toxic material was precipitated with methanol. Additional procedures resulted in the isolation of a pure crystalline substance, lycomarasmin, having the empirical formula $C_9H_{15}O_7N_3$ (22). Upon hydrolysis, lycomarasmin was found to yield glycine, aspartic acid,

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ammonia, and probably pyruvic acid (23). Other studies by this group involve the physiological action of this toxin, which in exceedingly small concentrations causes wilting of tomato leaves (3, 7, 8). These workers also sought to explain the nature of the mechanisms concerned in wilting and the relation of transpiration to the wilting process.

The present study is concerned with *Fusarium oxysporum* var. *nicotianae*, originally described by Johnson (15) as the cause of a wilt disease of tobacco. A proximate cause of wilting was sought by determining whether toxic metabolic products are elaborated by this organism, and, since this proved to be the case, efforts were made to learn something of the properties of the toxin.

MATERIALS AND METHODS

A culture of *Fusarium oxysporum* var. *nicotianae* was obtained from Dr. G. B. Lucas of the Tobacco Experiment Station, Oxford, N. C. The fungus was grown on a modified Richards' solution having the following composition: KNO_3, 10 gm.; KH_2PO_4, 5 gm.; $MgSO_4$, 2.5 gm.; $FeCl_3$, trace; glucose, 34 gm.; and distilled water, to make 1000 ml. Cultures were maintained at room temperature in 1-liter Erlenmeyer flasks containing 200 ml. of medium.

Young tobacco plants with 6–8 well-developed leaves were employed to assay the culture filtrates. The plants were grown in plant bands and when ready for use the roots were washed free of adhering soil particles, and the plants were then placed in 10-ml. vials with their roots submerged in the solutions to be tested.

Inasmuch as this study was preliminary in nature, undertaken to establish the possible existence of a wilt-inducing toxin in the fungal filtrates and to learn some of the chemical properties of the toxic material, environmental conditions under which the tests were made were not controlled, and the results are therefore qualitative or semiquantitative in character.

RESULTS

Fusarium oxysporum var. *nicotianae* grows well on Richards' solution, the mycelium forming a pink mat over the surface of the medium in about 10 days. The medium gradually becomes discolored owing to the secretion of metabolic products by the fungus.

Preliminary tests of the wilting power of filtrates from cultures of different ages disclosed that a growth period of at least one month is required before the filtrate has attained maximum toxicity. All subsequent experiments were performed using filtrates of cultures approximately one month old.

From 18 to 48 hours are required for the production of symptoms of toxicity in plants placed in the filtrates. Development of symptoms begins with the lowermost leaf, and progresses upward. The symptoms include a drooping and wilting of the leaves and stem tip, multiple small areas of necrosis in the laminar tissues, and collapse of vascular elements in the

proximal portions of the leaves. When wilted plants in incipient stages were placed in distilled water they failed to recover, indicating that such wilting is irreversible in character and must be regarded as pathological wilting.

Control plants placed in noninoculated Richards' solution were observed to become flaccid, a result in agreement with the findings of a number of previous investigators. Such plants recovered spontaneously within a few hours, however, and invariably recovered when transferred to distilled water. Such temporary wilting obviously is due to osmotic phenomena and must be differentiated from pathological wilting occurring when fungus toxins are present.

The filtrate was found capable of producing wilt and other associated symptoms when diluted 1 : 10, but not in greater dilution. When the filtrate was evaporated to dryness at 100° C., and the residue was redissolved in distilled water to restore the original volume, there was no apparent loss of toxicity. It may be concluded, therefore, that the toxic material is thermostable.

When the mycelial mat was macerated by grinding with quartz sand in a mortar and extracted in distilled water, the extract induced wilting of test plants. Further study was not made, however, of toxic substances within the mycelium.

When the filtrate was treated with an equal volume of 0.2 N $Ba(OH)_2$, a copious precipitate containing sulphate, phosphate, and presumably the salts of organic acids was formed. Because of its high salt content, and consequent possibility of causing wilting through osmotic action, this precipitate was not tested for toxic activity. The filtrate from the $Ba(OH)_2$ treatment, however, was evaporated to dryness *in vacuo* at 50°–60° C. Portions of the distillate were collected, and when tested proved completely non-toxic. The residue, when redissolved in water, appeared to be as toxic to test plants as the original filtrate. The toxic material is therefore non-volatile.

The pH of the culture solution, originally 4.6, became increasingly more alkaline, reaching 7.8 after growth of the organism for one month. Adjustment of the filtrate to pH 4.6 did not affect the production of toxic symptoms and indicates that alkalinity itself is not the cause of wilting.

After growth of *Fusarium oxysporum* var. *nicotianae* for one month, the filtrate gave no reaction with Fehling's solution or Benedict's solution, indicating that the glucose originally present had completely disappeared. An aldehyde does not seem responsible for the toxicity, and a test for ketones with Girard's reagent was likewise negative.

A test of the filtrate for nitrate with Trommsdorf's reagent was negative. A test for nitrite with sulphanilic acid and alpha-naphthylamine was also negative, indicating that nitrite, demonstrated by Rosen (24) to be the toxic agent in filtrates of *Fusarium vasinfectum*, is not responsible for the toxicity of filtrates of *F. oxysporum* var. *nicotianae*.

The filtrate gave a positive ninhydrin reaction with triketohydrindene hydrate, and a very strongly positive reaction for ammonium with Nessler's reagent. In view of the claims of toxicity of ammonium by Luz (19) and Elpidina (4), experiments were made to determine whether ammonium ions were responsible for toxicity of filtrates of *Fusarium oxysporum* var. *nicotianae*. Attempts to perform tests with filtrates treated with Nessler's reagent to remove ammonium failed, owing to toxicity of the Nessler's reagent itself, which induced severe vascular collapse in test plants. After the filtrate, which was alkaline in reaction, had been evaporated to dryness at 100° C., and the original volume had been restored with distilled water, a portion of it was tested with Nessler's reagent and was found to be negative, indicating that the ammonia had been completely volatilized. Another portion of this solution was found to have retained its toxic properties after removal of ammonia.

Since ammonium was not the toxic constituent, it was thought desirable to test for amines, in view of the results of Schaffnit and Lüdtke (25) and Lüdtke and Achmed (18). Determination of amino nitrogen in the filtrate, by the method of Van Slyke, gave the low value of 0.04 per cent. Further, the residue from the culture filtrate, when evaporated to dryness *in vacuo*, dissolved in a minimal quantity of distilled water, and tested with benzoyl chloride, failed to give the reaction characteristic of primary and secondary amines.

The possible protein nature of the toxic material was tested for the reason that certain well known bacterial toxins are known to be proteinaceous. The filtrate gave negative reactions with Millon's reagent and the xanthoproteic test, however. Further, an attempt to precipitate proteins from the filtrate by the addition of $(NH_4)_2SO_4$ equivalent to 4.2 M (including a correction for solution volume) gave no precipitate in 24 hours at a maintained temperature slightly above 0° C.

In order to test for the presence of lycomarasmin, the procedure of Plattner and Clauson-Kaas (22) was followed: After treatment of the filtrate with an equal volume of 0.2 N $Ba(OH)_2$ and filtration, the resulting filtrate was evaporated to dryness *in vacuo* at 50°–60° C., and the residue dissolved in the minimal amount of distilled water. The solution was then treated with charcoal, filtered, made acid to litmus, cooled in an ice bath to 0° C., and 3 volumes of absolute methyl alcohol were added. The material was then allowed to remain in a cold room for 24 hours after which the white flocculent precipitate was removed by filtration. When tested upon plants this precipitate was completely inactive. The aqueous-methyl alcohol filtrate was reduced in volume by vacuum distillation and shown to contain the toxic factor. The toxin of *Fusarium oxysporum* var. *nicotianae* is therefore not lycomarasmin nor any closely related polypeptide.

Three residues were prepared as follows: Culture filtrate, untreated, was evaporated to dryness *in vacuo*. To a second portion of culture filtrate was added an equal volume of 0.2 N $Ba(OH)_2$ to remove sulphate and phos-

phate, and after filtration the filtrate was evaporated to dryness *in vacuo*. Similarly, after precipitation with 0.2 N $Ba(OH)_2$, a third portion of filtrate was neutralized and then evaporated to dryness *in vacuo*. No apparent difference in the toxicity of these three residues was noted. It may be observed, however, that the alkaline filtrate yielded a hygroscopic dark brown residue, whereas the residue obtained after the addition of $Ba(OH)_2$ and neutralization was crystalline and possessed only a small amount of color.

Efforts were made to extract the toxic principle from the mixture of substances present in these residues through the use of various solvents. In these experiments the residue was repeatedly extracted with successive small volumes of the chosen solvent, the solvent was then removed by evaporation *in vacuo* and the soluble and insoluble portions were dissolved in distilled water and tested upon plants.

It was found that the toxin is insoluble in ether, and cannot be extracted by this solvent from the residue or from the original culture filtrate. Similarly, the toxic principle is insoluble in acetone. Toxic substances could be extracted from the residue, however, with methyl alcohol or with ethyl alcohol. With methyl alcohol, separation of a factor that induces wilting from one that induces necrotic symptoms could be obtained. The methyl alcohol-soluble portion produced severe wilting not accompanied by necrosis, while the fraction insoluble in methyl alcohol produced marked necrosis but only moderate wilting. This would appear to indicate that at least two toxic substances are metabolized by *Fusarium oxysporum* var. *nicotianae*.

DISCUSSION

The production of thermostable, nonvolatile substances toxic to plants appears to be a rather common characteristic of the pathogenic species of *Fusarium*. Because Richards' solution has so frequently been the medium chosen to demonstrate the elaboration of toxic materials by Fusaria, one wonders whether results obtained with cultures on this medium are really applicable to Fusarium diseases as they occur in plants in the field, and whether toxins are elaborated when the Fusaria are invading the vascular tissues. The demonstration of a toxin within the tissues of an infected plant (10) constitutes convincing evidence bearing on this phase of the problem, and indicates the need for similar studies with other species of *Fusarium*. The failure of Rosen (24) to obtain toxin from *F. vasinfectum* when grown in Uschinsky's solution or in nutrient broth indicated that the formation of toxins is conditioned by the composition of the medium. The need for studies of the effects of various carbon and nitrogen sources, minerals, and accessory growth factors upon toxin formation is apparent.

Another point, which has been noted by almost all workers, but to which apparently far too little significance has been attached, is that a prolonged period of growth, of the order of 4–6 weeks, is required for the formation of maximal amounts of toxin in cultures of Fusaria. Luz (19) states that the

substances most active in the induction of wilting appear to be related to the death of the mycelium. With the single exception of lycomarasmin, the toxins of Fusaria are not active in great dilution. One must infer, therefore, that they are produced in very small amounts, and must act over considerable periods in order to induce wilting in nature. In support of this conclusion, it may be recalled that tobacco plants, presumably invaded soon after transplanting in the field, may become almost mature before showing symptoms. In this disease, and presumably in other Fusarium diseases as well, the disease process must be characterized as chronic rather than acute.

All too little is known of the chemical nature of the toxins produced by Fusaria. The present results, admittedly inadequate, appear to indicate considerable similarity in the properties of the toxic materials produced by *Fusarium oxysporum* var. *nicotianae* with those produced by *F. vasinfectum* according to the results of Lüdtke and Achmed (18), and by *F. lini*, according to the work of Grossmann (12).

SUMMARY

When cultivated in a modified Richards' solution, *Fusarium oxysporum* var. *nicotianae* produces material which causes wilting and other symptoms of toxicity in tobacco plants. Both the culture filtrate and the fungus mycelium have toxic properties.

The chemistry of the toxic principle remains incompletely known. The toxin is thermostable and nonvolatile. Evidence is presented indicating that it is insoluble in ether or acetone, partially soluble in methyl alcohol and ethyl alcohol, and that it is not a nitrite, an ammonium salt, an aldehyde, a ketone, an amine, or a protein, and is not identical with lycomarasmin. More than one toxic factor appears to be involved, one of which is primarily concerned with wilting and the other with necrosis.

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QUANTITATIVE DETERMINATION OF TETRACHLORO-p-BENZOQUINONE ON TREATED SEED

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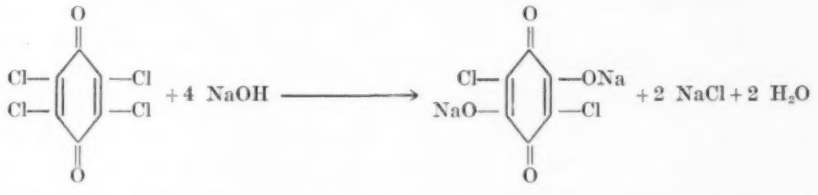
Simple, reliable methods for measuring the dosage of fungicidal protectants on seeds are needed both by scientists and practical agriculturists. The addition of a prescribed weight of chemical does not assure the desired dosage since much of the material may be lost on the treating equipment, by dispersion into the air, and by dislodgement in handling. Experience has shown that even the laboratory investigator who has rigid control over his methods frequently fails to secure complete, uniform coverage. An analysis of the treated seed for fungicide content would, therefore, contribute materially to the reliability of both the experimental and practical use of chemicals.

Since tetrachloro-para-benzoquinone (Spergon) has been recommended for use on pea (5, 6, 9, 10, 12), lima bean (1, 8), corn (5, 7, 9, 11) and other seed (2, 13) during the past six years, a quantitative method of analysis was considered desirable. This compound reacts with basic reagents to form highly colored substitution products. An effort was made to take advantage of this in developing a colorimetric method for the quantitative determination of tetrachlorobenzoquinone. The results of these studies and a new method of analysis are described in this paper. Data are presented on the reliability of the method when applied to treated seed of maize, peas, lima beans, beet, and spinach.

DESCRIPTION OF CHEMICAL PROCESSES

The best known reaction of tetrachlorobenzoquinone with basic materials is with aqueous sodium hydroxide to form the red-violet sodium chloranilate

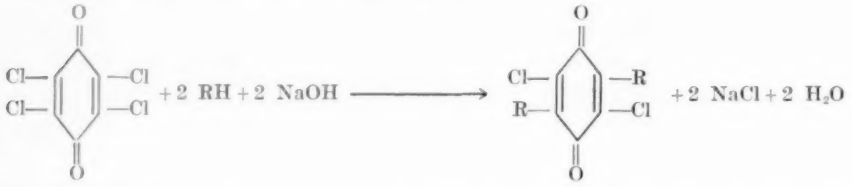


(3). Although the color is intense, the reaction is not satisfactory for use on treated seed. The hydrolysis of tetrachlorobenzoquinone is slow, and the caustic frequently removes natural pigments, particularly from maize seed. In order to avoid these difficulties it was necessary to first remove the tetrachlorobenzoquinone from the seed by use of a selective solvent. It

[1] The authors are deeply indebted to George E. O'Brien and H. P. C. Burrell for technical assistance in conducting these tests.

was found that three or four washings in acetone dissolved all of the material.

A method was developed, and used for some time, in which the acetone solution from 5 gm. of seed was made up to about 130 ml., treated with 50 ml. of 0.05 N sodium hydroxide, made to 250-ml. volume with acetone, and the optical density measured with a colorimeter. An intense yellow color develops which is proportional to the concentration of tetrachlorobenzoquinone. It is reasonable to assume that this yellow compound is an intermediate acetone condensation product since tetrachlorobenzoquinone is known to react with compounds such as malonic ester (14) and acetoacetic ester (4), that contain labile hydrogen:



This method is not satisfactory for general use because the yellow color is unstable and varies in intensity with slight differences in the quality of the acetone.

It was assumed that the fading was caused by hydrolysis; so studies were undertaken to eliminate water from the reaction. This was accomplished by substituting anhydrous diethylamine for the sodium hydroxide solution. The addition of this reagent immediately produced a yellow-green coloration that changed rapidly to an intense yellow. No further appreciable change in optical properties occurred during the next 15 minutes. The addition of a trace of water, however, caused rapid fading; so both the reagents and glassware must be free of moisture. Measurements made with a Beckman spectrophotometer showed that the yellow reaction product had an absorption maximum at about 450 mμ (Fig. 1).

Complete proof is not available on the nature of the reaction between tetrachlorobenzoquinone, acetone and diethylamine. The absorption spectrum does not agree with that of 2,5-bis (diethylamino)-3,6-dichloro-p-benzoquinone, the normally anticipated reaction product. It closely resembles the acetone condensation product described above and probably is identical to it. Regardless of the mechanism involved, the reaction seemed suitable for photometric analysis and was adapted to the determination of tetrachlorobenzoquinone.

MATERIALS AND METHODS

In order to determine the reliability of the acetone-diethylamine reaction for assaying tetrachlorobenzoquinone, a highly refined sample (M.P. 290°C), purified by chromatographic adsorption of the impurities on talc, was tested at different concentrations. Aliquots of 25, 20, 10, 5, and 2 ml. were transferred from a stock solution containing 0.25 gm. per liter to

50-ml. volumetric flasks and made up to about 40 ml. with acetone. Five ml. of anhydrous diethylamine was added and the mixtures made to volume with acetone. After standing for 3 min., the samples were placed in a Lumetron colorimeter equipped with matched 1-cm. absorption cells and

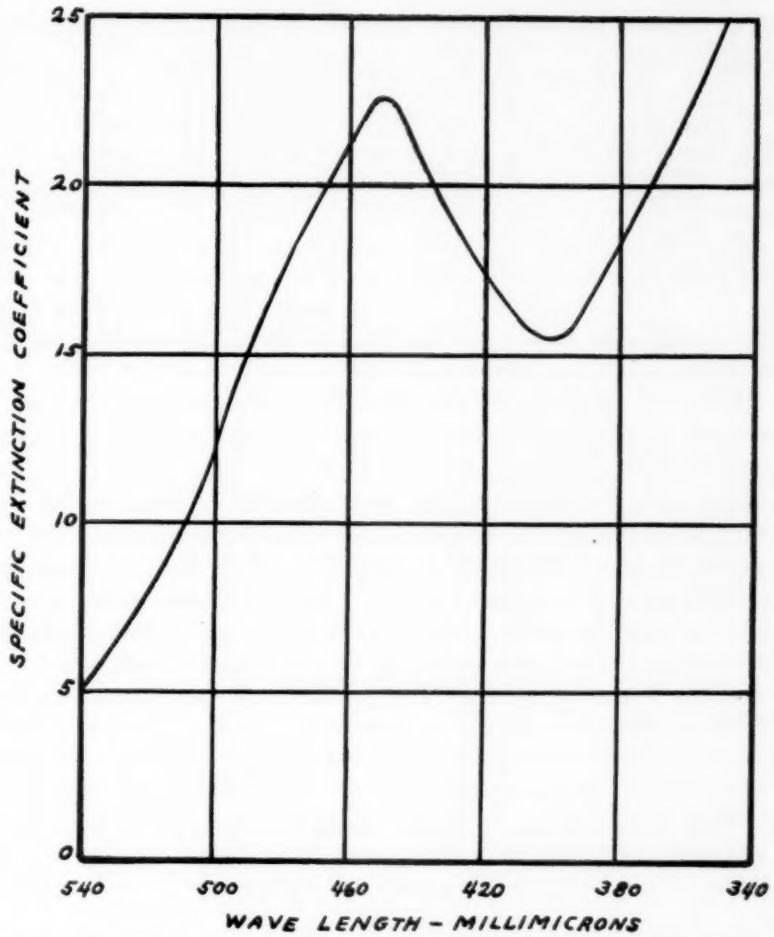


FIG. 1. Absorption spectrum of the reaction product of tetrachloro-p-benzoquinone with diethylamine and acetone.

a blue filter. The percentages of light transmitted (T) were measured against an acetone blank, converted to optical densities (log 100/T) and plotted against the concentration of tetrachlorobenzoquinone. A linear relationship was found that demonstrates adherence to the Beer-Lambert law. By application of the following formula, the apparent specific extinction coefficient (K) was calculated to be 17.3.

$$K = \frac{\log \frac{100}{T}}{G}.$$

where G equals the concentration of tetrachlorobenzoquinone in grams per liter in the final solution on which readings were taken.

The application of this method to treated seed was relatively simple. The amount of seed and the dilutions required were found to vary with the type of seed and dosage of fungicide. Preliminary tests indicated that the proportions outlined in table 1 were the most satisfactory. Large

TABLE 1.—*Preferred quantity of seed and dilutions used in colorimetric analysis*

Kind of seed to be analyzed	Minimum amount of seed required	Initial volume of solution	Size aliquot for analysis of seed bearing a dosage of				
			0.50 per cent	0.25 per cent	0.12 per cent[a]	0.06 per cent	0.03 per cent
	gm.	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*
Lima bean	25.0	250	2	5	10	25	25
Corn	5.0	50	2	5	10	25	25
Peas	5.0	50	2	5	10	25	25
Spinach	2.5	25	2	5	10	25	25
Beet	2.5	25	2	5	10	25	25

[a] Approximately one ounce per bushel of pea or corn seed.

smooth seed such as those of lima beans gave irregular results unless a relatively large sample was used; while small, rough seeds such as those of spinach gave consistent results with samples as small as 2.5 grams.

The following procedures were employed in determining the concentration of chemical on the treated seed. The seed were weighed to the nearest 0.01 gm. on a torsion balance. The tetrachlorobenzoquinone was dissolved by three successive washings in acetone (analytical reagent grade) and the combined extracts made to the volume recommended in table 1 for seed samples of different sizes. The solution was then filtered through a fluted No. 5 Whatman paper to remove dust and seed fragments. An aliquot whose size depended on the anticipated dosage of tetrachlorobenzoquinone (Table 1) was transferred to a volumetric flask and made up to about 40 ml. with acetone. Five ml. of diethylamine was added, the mixture made to volume with acetone and after 3 min. was placed in the colorimeter. The percentage transmission was measured against an acetone blank.

The dosage of tetrachlorobenzoquinone on the seed was calculated from the following equation in which Vo is the initial volume of solution, V the volume of the aliquot, T the percentage of light transmitted, W the weight of the seed, and K the apparent specific extinction coefficient of the reaction product.

$$\text{Percentage TCPB} = \frac{5\ \text{Vo}\log\frac{100}{\text{T}}}{\text{KVW}}$$

The applicability of the foregoing method was evaluated extensively on seed of hybrid dent corn and peas of the variety Perfection. Samples of 250 gm. of corn and 200 gm. of peas were weighed out to the nearest

0.01 gm. on a torsion balance (120-gm. capacity). These samples were placed in quart jars and sufficient chemical added to provide for dosages of 0.48, 0.24, 0.12, 0.06, and 0.03 per cent by weight after a nominal loss on the walls of the jar. The jars were then rotated on a vertical turntable for 30 min. at 28 r.p.m. to distribute the chemical uniformly on the seed. The samples were reweighed to determine the amount of fungicide adhering to them. Correction was made for any loss or gain in the untreated control, presumably due to moisture loss or uptake from the air.

The weighing of 200 grams or more of seed to detect 0.06 to 0.96 gm. of chemical is not so accurate as would be desired, since an error of 0.01 gm. may appear as 1 to 16 per cent error in dosage, depending upon the dosage level under investigation. Three different commercial preparations (Spergon, about 98 per cent active; Spergon-DDT-S1, about 96 per cent active; and wettable Spergon, about 48 per cent active) were applied at each dosage level to both types of seed.

Since discrepancies were anticipated in the data on dosages obtained by weighing and analysis, a third, independent measurement of dosage was sought as a guide and referee. Since the decay of seed in soils infested with *Pythium* sp. and *Rhizoctonia* sp. is directly affected by the dosage of chemical (9), 16 replications of 25 seeds each were sown in randomized block arrangement in greenhouse flats filled with naturally infested composted soil. The seed was covered to a uniform depth, watered heavily, placed in a cabinet at 55° F. for seven days and then removed to a greenhouse at 65° to 80° F. Records on emergence were taken 10 days later and subjected to analyses of variance.

EXPERIMENTAL RESULTS

Data obtained on the dosage of fungicide on the various samples of corn and pea seed by analytical and weighing methods are presented in table 2. The results by the two methods are in strikingly close agreement. Divergence at the highest dosage ranged from 0 to 8 per cent while at the lowest dosage the discrepancies ranged from 0 to 30 per cent of the total chemical. This latter figure is little more than would be expected from the error inherent in the weighing method.

The response of seed treated with different dosages to decay fungi was very uniform within each series of chemicals and between chemicals when allowance is made for differences in the percentages of active ingredient. All the analytical data have been corrected to account for the inert diluents and conditioning agents present in the formulations.

The analytical data in table 2 are the means of three separate determinations on 5-gm. samples of seed. The average range of these triplicate tests was 5.64 per cent of the total chemical present. The standard deviation for single determinations was 3.3 per cent of the chemical. This compares to a standard deviation of 1.3 per cent for the analytical constant obtained on a stock solution of the pure chemical. It follows that the

errors due to variations in extraction and distribution of chemical on the seed must be in the order of 3.05 per cent. Furthermore, it is obvious that a method embodying such precision would not lead to discrepancies

TABLE 2.—*Comparative dosage of Spergon on corn and pea seed determined by weighing and colorimetric analysis*

Treatment applied		Dosage on corn[a]		Emergence of corn	Dosage on peas[a]		Emergence of peas
Material used	Desired dosage	By weight	By analysis		By weight	By analysis	
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Spergon	0.48	0.46	0.461	86.8	0.45	0.455	91.0
	0.24	0.26	0.256	84.5	0.22	0.240	89.2
	0.12	0.12	0.140	75.5	0.12	0.132	70.2
	0.06	0.06	0.071	68.5	0.05	0.058	58.5
	0.03	0.04	0.038	68.5	0.04	0.036	60.5
Spergon–DDT	0.48	0.47	0.502	83.0	0.44	0.486	86.2
	0.24	0.26	0.279	77.0	0.21	0.234	83.8
	0.12	0.12	0.135	74.3	0.12	0.134	80.5
	0.06	0.06	0.071	67.8	0.05	0.061	61.8
	0.03	0.03	0.037	61.5	0.04	0.038	54.8
Spergon–Wettable	0.48	0.48	0.445	79.3	0.44	0.412	84.2
	0.24	0.26	0.238	74.0	0.22	0.215	76.2
	0.12	0.12	0.115	66.3	0.12	0.117	66.2
	0.06	0.06	0.060	58.8	0.05	0.056	47.5
	0.03	0.03	0.028	48.5	0.03	0.029	51.2
None	.....	0.00	0.000	15.8	0.00	0.000	37.5
None	.....	0.00	0.000	16.0	0.00	0.000	35.8
None	.....	0.00	0.000	18.0	0.00	0.000	34.2
Minimum Sign. Diff. at 5 per cent				6.4			7.9
Minimum Sign. Diff. at 1 per cent				8.4			10.5

[a] Dosages determined by analytical method corrected for percentage of active ingredients based on triplicate analyses.

as large as those between the results of weighing and analyses given in table 2. The deviations are primarily attributable to errors in the weighing of large samples of seed to 0.01 gm.

The practicability of the colorimetric method was tested to a more limited extent on seed of lima beans (var. Fordhook), spinach (var. Heavy Pack), and beets (var. Asgrow Canner). Seed were treated at the rate ordinarily used and the dosage determined by reweighing. The colorimet-

TABLE 3.—*Results of analysis of Spergon on beet, lima bean, and spinach seed*

Type of seed treated	Desired dosage of Spergon	Calculated dosage	
		By weighing	By analysis[a]
	Per cent	*Per cent*	*Per cent*
Lima beans	0.22	0.22	0.21
Spinach	0.50	0.51	0.50
Beet	1.00	0.95	0.92

[a] Average of three analyses, corrected for percentage of active ingredient.

ric analyses were conducted as described for corn and peas except that the size of samples was varied, as indicated in table 1, and a minor correction was necessary for pigments extracted from beets. This was made by extracting untreated seed in acetone and measuring the absorption of the solution in the presence of diethylamine. The data presented in table 3 show that the analytical method was applicable to these seed.

DISCUSSION

Although the method described in this paper is convenient and reliable, certain precautions must be observed in its use. For most purposes, satisfactory results can be obtained from duplicate samples. In the tests under discussion 10 to 20 seed (5 gm.) of either peas or corn were representative of the entire population. It is entirely possible, however, that larger samples may be required for poorly treated seed where the chemical is not so uniformly distributed. If larger samples are used a proportional increase in the amount of acetone will be necessary.

In adapting this method to commercial use where a minimum of calculations may be preferred, the following procedure can be substituted. A carefully weighed sample of the commercial product to be applied to the seed should be dissolved in acetone and diluted to a range of concentrations. These samples are used for standardizing the available equipment. The readings obtained from colorimeters that record data in optical densities can be plotted on rectangular graph paper against the known concentrations of chemical and the best straight line drawn through these points. Data from instruments recording percentage of light transmitted must be plotted on semi-logarithmic paper in order to obtain a straight line. Readings from unknown samples can be quickly transcribed to milligrams of chemical directly from these graphs.

SUMMARY

A simple colorimetric method of analyzing seed for tetrachloro-para-benzoquinone has proved exceptionally reliable on pea, corn, lima bean, spinach, and beet seed treated at various dosage levels over a range of one-fourth to four times the recommended concentration. The method consists of washing the chemical from the seed with acetone, adding diethylamine, and measuring the amount of light absorbed by the yellow reaction product that is formed. The optical density is directly proportional to the amount of tetrachloro-para-benzoquinone dissolved from the seed.

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PHYTOPATHOLOGICAL NOTES

A Valsa Associated with Cytospora Canker of Spruces.[1]—A canker disease of spruces was described by Gilgut and Boyd[2] in 1933. The causal fungus was identified tentatively by Dr. L. E. Wehmeyer,[3] as *Cytospora Kunzei* Sacc. No perfect stage of the fungus was observed on spruce.

Recently a species of *Valsa* was found associated with cankers on Colorado blue spruce (*Picea pungens* Engelm. var. *glauca* Beissm.), and Norway spruce (*Picea abies* [L.] Karst.). The writer believed the fungus to be *Valsa Kunzei* Fr., the perfect stage of *Cytospora Kunzei* Sacc., which has been reported as the cause of the spruce canker disease. A specimen was sent to Dr. L. E. Wehmeyer at the University of Michigan who stated that the fungus is the Leucostoma type of *Valsa,* which he has called *Valsa Kunzei* Fr.

The perithecial stromata of *Valsa Kunzei* which occur on cankered spruce branches are fairly widely scattered, rarely confluent, often interspersed with pycnidial stromata. They are seated in the cortex, are circular to fusoid, pustulate, erumpent, greyish-black, and measure 1.5–2.5 mm. in diameter. The ectostroma is truncate conical, and is surmounted by a greyish disc which is 0.5–.75 mm. in diameter and bears numerous black ostioles.

The perithecia, 7–20 in number, are seated closely together in the entostroma. They are globose, leathery, yellowish to black, with long converging necks. Exclusive of the necks the perithecia measure 431–631 × 447–616 μ. The necks measure 92–169 × 616–1032 μ, and are lined with delicate hyphae. Paraphyses are lacking.

The asci are numerous, broad-clavate, sessile or subsessile, hyaline, eight spored, and measure 5–7 × 20–30 μ.

The ascospores are biseriate or bunched in the ascus, allantoid, hyaline, and measure 2.5 × 5–7 μ.

Upon germination on potato-dextrose agar the spores increase to an average size of 12 × 16.5 μ. They become nearly oval, oblong, or sometimes dumbbell-shaped. Two or more oil droplets are usually visible. Germ tubes are formed at both ends of most spores; less frequently one or three tubes are formed. The hyphae are hyaline and septated at unequal intervals. Irregular branching of the hyphae begins 18–24 hours after germ-tube formation.

Single-ascus and single-ascospore cultures on potato-dextrose agar and spruce-decoction agar were compared with pycnospore cultures of the *Cytospora* which causes the spruce canker disease. The ascospores and the

[1] Part of a thesis submitted in partial fulfillment of the requirements for the Degree of Master of Science at the University of Massachusetts. The investigations, directed by Professor A. Vincent Osmun, were conducted under a grant for research in the Department of Botany from the F. A. Bartlett Tree Expert Company, Stamford, Connecticut. Published as Contribution No. 646 of the Massachusetts Agricultural Experiment Station.

[2] Gilgut, C. J., and O. C. Boyd. Cytospora canker of *Picea* spp. Phytopath. 23: 11. 1933.

[3] Gilgut, C. J. Cytospora canker of spruces. Proc. Natl. Shade Tree Conf. 12: 113–118. 1936.

pycnospores both gave rise to a dull white or cream-colored, appressed mycelium, roughly circular or with fans, and with an irregular-crenate margin. Faint concentric zones of light and darker shades were formed. Considerable variation in coloring occurred on the same medium. Seven to ten days after inoculation numerous minute olivaceous bodies were formed on the surface of the medium. Twenty-five to 30 days after inoculation some of these bodies had enlarged to 1 mm. or more in diameter. Cross sections showed them to be rudimentary pycnidia, each consisting of a firm black center, tufted with grey hyphae, and containing hyaline, allantoid spores. None of the pycnidia matured enough to expel spore cirri.

The growth and appearance of the *Valsa* and the *Cytospora* were nearly identical on steam-sterilized twigs of Colorado blue spruce and Norway spruce. The mycelia grew from the cut ends and from the needle cushions in mats of white hyphae. Twenty-five to 30 days after inoculation superficial, globose, greyish pycnidia had been formed which bore hyaline, allantoid pycnospores, 2×6–$7\ \mu$. Mycelial growth was slightly more luxuriant on Colorado blue spruce than on Norway spruce; more and slightly larger pycnidia developed on those twigs inoculated with the *Cytospora* than on those inoculated with the *Valsa*.

The pathogenicity of the *Valsa* was tested by inoculating five-year-old Norway spruce trees in the greenhouse. On April 24, 1947, ten lateral branches and one trunk were inoculated with mycelium from single-ascospore cultures. For each inoculation a razor cut was made in the bark and the mycelium placed therein. The inoculations were wrapped with moist cotton and waxed paper. The wrappings were left intact for one week. Six control inoculations with sterile agar were made in the same manner. On July 29, 1947, five of the inoculated branches were dead, each having been girdled by a resinous canker at the point of inoculation. Two other inoculated branches were cankered but alive. The remaining three were healthy with no signs of cankers. In the case of the trunk inoculation resinosus occurred, and a canker had been produced which extended halfway around the tree. No ill effects were apparent in the crown of this tree. No fruiting bodies had been formed on any of the inoculated trees. The fungus was re-isolated from each of the cankered branches. All of the control branches remained healthy and the razor wounds had healed.

Although a positive relationship was not established between the species of *Cytospora* which causes the canker disease of spruces and the species of *Valsa* which was found associated with cankers on spruce, the similarity in growth characteristics on nutrient media and sterilized twigs indicates that the *Valsa* may be the perfect stage of the *Cytospora*. Limited experiments have shown that cankers and death of Norway spruce branches may follow inoculation with mycelium from single-spore cultures of the *Valsa*. However, sufficient fertile perithecia were not found to determine the importance of the ascospores in the natural spread of the disease.—DAVID H. MARSDEN, Massachusetts Agricultural Experiment Station, Amherst, Massachusetts.

Low Temperature As a Factor in the Germination of Dwarf Bunt Chlamydospores.[1]—One of the characteristics of dwarf bunt (*Tilletia caries* (DC.) Tul.) of winter wheat is the difficulty encountered when trying to induce the chlamydospores to germinate in the laboratory.[2] Investigations[3] have shown that spores of this fungus may be induced to germinate by soaking them in water for several months prior to incubation on water agar.

Up to 30 per cent germination has been obtained by this method when the spores were incubated at 10° C., but no germination has been observed at higher temperatures. This fact, and the fact that the dwarf-bunt fungus attacks only wheat planted in the fall, suggested the possibility that the spores require a still lower temperature for germination. Investigations in line with this hypothesis were carried out and are reported here.

The materials used are from collections of dwarf bunt, from the Pacific Northwest,[4] which were stored at room temperature for periods ranging from 3 to 13 years. All germination tests reported were made in 1947, taking spores directly from storage without any pre-soaking.

Germination tests were made on 4 per cent water agar in Petri dishes at temperatures of 0°, 5°, and 10° C. Observations were made weekly to determine the progress of germination. Percentages of germination after 49 days were determined by estimate while those on later dates were determined by actual count. No attempt was made to determine the percentage of germination after 75 per cent of the spores had germinated because by then the colonies were too closely intermingled. The experiment was discontinued after 104 days, as further increases in germination could not be detected after that time.

The results of this test are presented in table 1. These data indicate that the optimum temperature for germination was 5° C. Germination began at the end of the fifth week. Percentage germination determinations, however, were made only at the three different times, as shown in table 1.

At the end of 49 days up to 50 per cent germination had occurred in all collections, and this increased to 75 per cent at the end of 70 days. The period of germination, therefore, extended over about five weeks, whereas in common bunt it extends over a period of but a few days.

In these tests no germination was observed at 10° C. In some other tests a high percentage germination was obtained at 0° C., after a period of three months. The results of all tests indicate that the optimum temperature for spore germination in dwarf bunt under the conditions described is approximately 5° C. When incubated a few degrees above or below this

[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture and the Division of Plant Pathology, Washington Agricultural Experiment Stations. Published with the approval of the Director as Technical Paper No. 750.

[2] Holton, C. S. Preliminary investigations on dwarf bunt of wheat. Phytopath. 31: 74–82. 1941.

[3] Holton, C. S. Chlamydospore germination in the fungus causing dwarf bunt of wheat. Phytopath. 33: 732–735. 1943.

[4] All materials used in these tests were obtained from Dr. C. S. Holton, to whom the writer is indebted for aid and encouragement during these and related studies.

temperature, there seems to be a marked reduction in both the percentage and the speed of germination. In preliminary tests of several 1947 collections of dwarf bunt spores, good germination was observed under the same

TABLE 1.—*The percentage germination of dwarf-bunt chlamydospores after three different periods of incubation on 4 per cent water agar at three different temperatures, Centigrade.*

Place and year collected	After 49 days			After 70 days			After 104 days		
	0°	5°	10°	0°	5°	10°	0°	5°	10°
Plummer, Idaho—1938	0	25–50	0	0	75	0	10	75	0
Cache valley, Utah—1934	0	25–50	0	0	75	0	2	75	0
Cache valley, Utah—1944	0	10–25	0	0	50	0	3	50	0
Nez Perce, Idaho—1937	0	25–50	0	0	75	0	3	75	0
Troy, Idaho—1940	0	25–50	0	0	40	0	2	50	0
Troy, Idaho—1944	1–5	25–50	0	10	75	0	20	75	0
High Prairie, Wash.—1940	0	25–50	0	0	75	0	2	75	0
Kalispell, Montana—1942	0	25–50	0	3	60	0	40	75	0

conditions, indicating that age is not a factor in the germination of these spores.—CONLEY V. LOWTHER, Department of Plant Pathology, Washington State College, Pullman, Washington.

Ringspot of Papaya (Carica papaya) in the Hawaiian Islands.—Papaya ringspot, a previously undescribed disease, was discovered in the Territory of Hawaii at Kailua, Oahu, in March, 1945. Its occurrence and general symptoms were recorded by Lindner *et al.*[1] Subsequently, experiments reported by Jensen[2] demonstrated that papaya ringspot is caused by a virus which is transmissible by the aphid *Myzus persicae* (Sulzer). In these experiments symptoms developed in papayas in from 11 to 21 days after inoculation.

The most distinctive and reliable symptom of the disease appears on the fruits as they approach maturity. The surfaces of such fruits, while still mostly green, develop yellow rings with green centers. The rings vary from $\frac{1}{8}$ to $\frac{3}{4}$ of an inch in diameter.

Foliage symptoms of the disease include elevation, or puckering, of the leaf tissue between the veins and veinlets of the youngest leaves and irregular mosaic patterns in expanded leaves. The mosaic symptoms are most pronounced in field trees during the winter season, becoming masked in varying degrees during the summer.

Because papaya ringspot produces typical mosaic symptoms in the foliage, the disease could appropriately be called papaya mosaic. However, the name papaya mosaic has already been used for another papaya disease in Hawaii which Parris[3] reported to be due to a virus. Moreover, Ho and Li[4]

[1] Lindner, R. C., D. D. Jensen, and W. Ikeda. Ringspot: New papaya plunderer. Hawaii Farm and Home 8(10): 10, 12, 14. 1945.

[2] Jensen, D. D. Virus diseases of plants and their insect vectors with special reference to Hawaii. Proc. Hawaiian Entomol. Soc. 12: 535–610. 1946.

[3] Parris, G. K. A new disease of papaya in Hawaii. Proc. Amer. Soc. Hort. Sci. 36: 263–265. 1938.

have applied the name papaya mosaic to a virus disease of papayas in China, and Baker[5] has used the term papaw mosaic for a virus disease of papayas in Trinidad.

The origin of papaya ringspot is not known. Its restricted distribution, despite rapid spread within plantings that have become infected, seems to indicate that the disease, in its present form, became established in the Territory within recent years.

Papaya ringspot virus rarely, or perhaps never, kills infected papaya plants. However, the disease appears to retard the growth of plants and weaken them. In areas of high disease incidence commercial papaya production has been appreciably affected.

A survey of the island of Oahu was made in the spring of 1945, in cooperation with the Board of Agriculture and Forestry to determine the distribution of papaya-ringspot disease. The disease was found to be limited to a relatively small portion of the island. The Kailua-Maunawili district, near the eastern end of the island, was found to have a fairly high percentage of diseased papaya plants. On one farm, nearly 100 per cent of the papayas were affected. The incidence of disease was observed to diminish progressively to a 20 per cent level within a half-mile of this point. The distribution from this heavily infested area was mostly to leeward (*i.e.*, to the southwest). Five scattered infestations were found in the Kokokahi district, about 3 miles to the north of the heavily infested Kailua district. In two of these, the papaya ringspot could be traced to use of papaya seedlings originally grown in the Kailua area and subsequently transported to the Kokokahi district for planting. Diseased plants were also found scattered throughout the city of Honolulu, which is directly to the leeward side of the Kailua area, but across the Koolau range of mountains that vary in this region from 1200 to 3100 feet in elevation.

Spread of disease within individual plantings proved rapid during the winter and spring months, but slow during the summer months. This is presumed to be a result of seasonal abundance of aphid vectors.

During the latter part of 1945 the disease progressed rapidly in the direction of prevailing winds within the Kailua-Maunawili district but there has been little lateral spread. In the spring of 1946 the area of essentially complete infection had increased from the one farm to include a number of others within a half-mile radius with proportionate increases in the area immediately to the southwest.

The possible existence of papaya ringspot on islands of the Territory other than Oahu was investigated during June and July of 1946, when a survey of Kauai, Maui, Molokai, and Hawaii was made. Principal attention was given to climatologically diverse areas where papayas had been culti-

[4] Ho, W. T. H., and L. Y. Li. A virus disease of papaya (Carica papaya L.). Preliminary notes on the virus diseases of some economic plants in Kwangtung Province. Lingnan Sci. Jour. **15**: 67–78. 1936.

[5] Baker, R. E. D. Papaw mosaic disease. Trop. Agr. (Trinidad) **16**: 159–163. 1939.

vated for long periods of time in commercial and in dooryard plantings. Representative plantings within the immediate vicinity of airports and seaports were given special consideration since the introduction of a new disease would be especially likely in such areas. In no part of any of the four islands included in this survey was the disease found.

The possibility of control of papaya ringspot, as it occurs in the Territory of Hawaii, would seem to be dependent on the applicability of one or more of four commonly used procedures. These are (1) control of insect vectors, (2) substitution of immune or highly tolerant varieties of papaya for those now grown, (3) interpolation of crop-free periods, and (4) destruction of diseased trees.

Control of insect vectors would mean control of aphids, especially of the aphid *Myzus persicae,* in the case of papaya ringspot. Aphicides are effective but expensive and not commonly used by papaya growers in Hawaii. Moreover, *Myzus persicae* breeds on many species of plants and would tend to reinfest papaya soon after treatment of this plant.

Substitution of immune or highly tolerant varieties of papaya for those now grown offers no immediate prospect of solving the problem. At present no variety immune from the disease is known. About 15 varieties, varietal strains, and hybrids, representing material from both local and foreign sources, have been grown in areas where the disease prevails. In every instance, this material has contracted the disease through natural spread.

Crop-free periods of the usual seasonal type would be inapplicable to the problem of control in the case of a papaya disease, because the host is grown as a short-lived perennial in overlapping succession. It would be necessary to induce growers to omit succession planting for a period long enough to provide a wholly crop-free interval.

Destruction of diseased trees sometimes seems uneconomical to owners, who may prefer to continue picking fruit as it matures rather than attempt control by eradicating the reservoir of disease within the planting. Control by removal of diseased trees, however, would be favored by many circumstances. Not enough trees are involved by disease, as yet, to affect appreciably the current supply of fruits for market. The papaya plant is readily cut down and does not regenerate from roots left in the ground if all stem tissues are removed by the initial cut. It rarely grows as a weed in the Territory but is essentially confined to cultivated plantings and to dooryard plots, where it is readily found. Thus far no other species of plant has been found capable of acting as host of the causative virus.

In view of the considerations discussed above, removal of diseased trees would seem to provide the best basis for a program for control of papaya-ringspot disease, other procedures being considered as of possible supplementary value whenever applicable.—F. O. HOLMES, The Rockefeller Institute for Medical Research, Princeton, N. J.; J. W. HENDRIX, W. IKEDA, D. D. JENSEN, R. C. LINDNER, and W. B. STOREY, University of Hawaii Agricultural Experiment Station, Honolulu, T. H.

REPORT AND ABSTRACTS OF THE SECOND ANNUAL MEETING OF THE NORTHEASTERN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The second annual meeting of the Northeastern Division of the American Phytopathological Society was held in Poughkeepsie, New York, November 25–26, 1947, immediately following the New England–New York Spray Specialists' Conference. Approximately 70 persons attended the meeting. Discussion meetings were held on the following topics: 1947 Cooperative Fungicide Tests for Apple Scab Control, Classifying Scientific Data for Punch Cards, Turf Diseases and Their Control, Fungicide Control of Potato Diseases, and Diagnosis of Plant Diseases. Ten formal papers were presented at one afternoon session. At a short business meeting the new constitution was adopted. The following officers were elected for 1948:

President: O. C. Boyd
Vice President: D. H. Palmiter
Secretary-Treasurer: L. M. Black
Councilor: S. E. A. McCallan

ABSTRACTS OF PAPERS PRESENTED AT THE MEETING

Relation of insecticides to the development of diseases of ornamentals. A. W. DIMOCK. The development of leafspot (*Septoria obesa*) in the upper foliage of chrysanthemums was observed under conditions which precluded spore dissemination by splashed water, the only efficient dispersal mechanism then recognized. In all cases the plants had been sprayed with rotenone insecticides. Viability tests of spores suspended briefly in spraying-strength emulsions of such insecticides, and actual inoculations with the suspensions, proved that viable inoculum could be spread from infected basal leaves to the upper foliage by such sprays. Of 11 materials tested, some showed very high, others very low fungicidal value. Although spray droplets of such suspensions may evaporate too rapidly to permit immediate infection, this may occur subsequently as a consequence of nightly deposition of dew. An unusual epidemic of blackspot (*Diplocarpon rosae*) developing abundantly on the upper, but as only a trace on the lower, foliage of greenhouse roses was observed. Spores had been disseminated to the upper foliage by syringing with water for red spider control, though the water had evaporated too rapidly for infection to occur. A subsequent application of tartar emetic and sugar, fogged on the upper leaves for thrips control, provided a hygroscopic deposit retaining sufficient condensed moisture long enough to permit infection.

Common blight of bean as a screen for testing chemotherapeutic activity. A. E. DIMOND and E. M. STODDARD. Common blight of bean (*Xanthomonas phaseoli*) was found to be a good medium in screening chemicals for therapeutic activity. Systemic infections produced sharp differences between good treatments and checks. Red Kidney beans were grown in sand and fertilized to give maximum symptoms of disease. Systemic infection was produced by inoculating the stem with bacterial stabs. A solution of the chemical to be tested, just non-phytotoxic in concentration, was applied to the potted plants on the nine days following the inoculation period. Two weeks after inoculation plants were rated for incidence of disease as measured by percentage of infection, and severity of symptoms as measured by a disease index. Typical data for percentage of infection and disease index follow: $KMnO_4$—100 and 3.48; $CaCl_2$—86 and 1.40; $ZnSO_4$—61 and 1.17; Hydroquinone—89 and 2.05; Auramine—58 and 0.57; Salicylic acid—14 and 0.17; Check—100 and 3.38. Tests so far conducted indicated greater success when chemicals were applied after rather than before inoculation.

Evaluation of organic fungicides for the control of Venturia inaequalis (Cke.) Wint. J. M. HAMILTON. Most organic fungicides have given mediocre or erratic performance in field tests. The testing of organics on potted greenhouse-grown trees as to retention and eradicative action at the time infection occurs has aided in the interpretation of their performance. Puratized Agricultural Spray (phenyl mercuri triethanol ammonium lactate), 806 (phenyl mercury compound), and PMAS (phenyl mercury salt) were comparable to liquid lime-sulfur in the length of time that they controlled scab after infection began. Phygon (2,3-dichloro-1,4-naphthoquinone), copper 8-quinolinolate, and manganese ethylene bis dithiocarbamate were moderately effective in the order of their inherent toxicity. The presence of moisture to release the toxicants after their applica-

tion markedly increased their effectiveness. This was not true with the Puratized group. Fermate (ferric dimethyl dithiocarbamate), Dithane Z78 (zinc ethylene bis dithiocarbamate), and the glyoxalidine materials were not eradicative and functioned mainly as preventives. Good-rite P.E.P.S. (polyethylene polysulphide) was adhesive but did not redistribute. The work supported the trend in field data in that the organics with the greatest retention were superior in seasons where sprays could be timed. On the other hand, eradicative type materials were most effective in periods of more or less continuous precipitation. A mixture of an eradicative material with a protective fungicide has merit.

Factors influencing the control of carrot yellows with DDT. G. E. R. HERVEY and W. T. SCHROEDER. The vector of carrot yellows in western New York, *Macrosteles divisus* (Uhl.), occurs and breeds on carrots from the time the plant emerges until harvest. In 1946 and 1947 there was a definite peak in vector population, beginning when the carrots were three to five inches tall and extending for 25 to 30 days. This peak resulted almost exclusively from migrating adults. The possibility that factors other than vector population may influence the amount of yellows was indicated by the fact that in comparable nontreated plots, the leafhopper population in 1946 was three times as great as in 1947, yet the disease was slightly higher the latter year. DDT applied during the peak immediately reduced the insect population to practically nil, but it built up rapidly by migration. Dusts were less effective than sprays, and the latter were more effective when used with an adhesive. Plots treated with DDT showed no nymphal development for as much as 8 weeks after the last application. DDT applications timed to coincide with the beginning of the peak in vector population gave as good or better control with fewer applications than when this factor was ignored.

Elimination of spotted wilt from a stock of dahlia. FRANCIS O. HOLMES. An unusually severe outbreak of spotted wilt in dahlia occurred near Vineland, New Jersey, during 1946. The Rhythm dahlia was most severely affected of all varieties observed, and every available plant was diseased. In this dahlia, as in all others, lower leaves were more damaged than upper ones. Shoots grown from diseased roots generally appeared healthy at first, though if left attached to the old roots they showed lesions of the disease eventually. Tip cuttings taken from emerging shoots were readily rooted. Only a few of these showed disease when established as potted plants. A majority of the rooted tip-cuttings remained healthy in appearance and were grown to blossoming in the greenhouse under spring season conditions without showing any evidence of spotted wilt. Thirty of the apparently healthy cutting-plants also grew vigorously throughout the summer in a field plot. In no case did the disease reappear. It was concluded that this stock of Rhythm dahlia had been freed of virus by radical removal of diseased tissues, *i.e.*, old roots, stems, and lower leaves.

A comparison of aircraft dusting and ground spraying for the control of tomato diseases. G. L. MACK and W. T. SCHROEDER. Six alternate applications of Zerlate (zinc dimethyl dithiocarbamate) and Tribasic copper sulfate (54 per cent) were applied to tomatoes as dusts with a Bell helicopter and with a Stearman fixed wing aircraft, and as sprays with a trailer-drawn brush-boom spray rig. Application rates were adjusted to deliver 4 lb. Zerlate or 4 lb. metallic copper per acre. Ten-row plots, each approximately 1.7 acres in area and separated from each other by sprayed five-row buffer plots were randomized in each of three blocks. Data were obtained only from the five center rows of each plot. Following the fourth application, deposition and distribution of copper on the upper and lower surfaces of twenty leaves, selected at random from each plot at three different positions on the plant were determined by a print method and analyzed statistically. Coefficients of variability indicated a much more even distribution of the fungicide with the ground sprayer than with either of the aircraft applicators. Copper deposit ratings were related significantly to total yield and late blight control. Significant differences between the three applicators in yield and late blight control ranked the ground sprayer first, followed by the helicopter and aeroplane. Anthracnose control was significantly better with the ground sprayer than with either of the aircraft applicators.

Apple scab control with elemental sulfurs in the Hudson Valley in 1947. W. D. MILLS and J. D. VANGELUWE. Six formulations of air-ground sulfurs were compared with Magnetic "70" sulfur paste, Troy Flotation sulfur paste, and micronized sulfur, using 2½ lb. actual sulfur per 100 gal. on McIntosh and Delicious varieties. One formula of air-ground sulfur was compared with the paste sulfurs and micronized sulfur at 5 lb. actual sulfur. Sprays were timed by bud development and rain forecasts. Two of the applications were made at the beginning of infection period rains. Treatments at 2½ lb. per 100 gal. were remarkably good in view of the heavy rains between sprays. The two paste types of sulfur were equally effective. Both pastes and one formulation of air-

ground sulfur were significantly better (99: 1) in fruit scab control than micronized sulfur when 5 lb. actual sulfur were used on both McIntosh and Delicious. The control of fruit scab on Delicious by 1 and 1½ lb. Fermate (ferric dimethyl dithiocarbamate) equalled that obtained with the 5-lb. dosage of the better elemental sulfurs, and was significantly better (99: 1) than micronized sulfur.

Some effects of fungicides on yield, quality, and maturity of apples in 1947. D. H. PALMITER. Phygon (2,3-dichloro-1,4-naphthoquinone)—¾ lb. Phygon—½ lb. plus Goodrite P.E.P.S. (polyethylene polysulphide)—1 lb., Puratized Agricultural Spray (phenyl mercuri triethanol ammonium lactate)—1 pt., Fermate (ferric dimethyl dithiocarbamate)—1½ lb., copper 8-quinolinolate—1 lb., Micronized Sulfur—5 lb., and 341C (2-heptadecyl glyoxalidine)—1 qt., each in 100 gal. water and applied to McIntosh trees in seven sprays, held fruit infection from apple scab to less than five per cent. Manganese ethylene bis dithiocarbamate at 1½ lb. and P.E.P.S. at 2 lb. per 100 gal. did not afford as good control as the other materials. DN–111 (dicyclohexyl amine salt of dinitro-orthocyclohexylphenol) severely injured the foliage and caused fruit drop when applied one month after an application of Phygon. Trees sprayed with 341C showed foliage injury in the form of purple veins and a reduction in leaf size and color. Fruits from these trees were smaller than average. Trees in the Puratized plots matured their fruit a week later than the rest of the orchard and had the best color and quality. Russet injury on similarly sprayed Greening plots was very severe with 341C, moderate with Sulfur, Fermate, and P.E.P.S., slight with copper 8-quinolinolate and Puratized, and almost absent with Phygon and Manganese carbamate.

Air blast application of oil-soluble fungicides to row crops. J. B. ROWELL and F. L. HOWARD. Modern air blast methods of pesticide application require a reduced quantity of carrier. Likewise, fungicides compatible with the new oil-soluble insecticides are needed for simultaneous application. Quantitative data on the specifications needed for implementing this principle of disease control were obtained by experimenting with a modified Potts-Spencer "Mist Blower" mounted on a Bolens HiBoy row-crop two-wheel tractor. Particle size and type of oil are of major importance in safeness on potato and tomato foliage. A wide distribution pattern induced by turbulence is essential for uniformity of coverage and avoidance of injury. Oil-soluble fungicides were applied with slight or no injury to field plots of potato, tomato, cucumber, and snap bean. Bean anthracnose control was demonstrated with Mycotox–4 (substituted phenyl ester) and Onyx DL–1 (didodecyl dimethyl ammonium bromide) carried in 3 gallons of Shell Horticultural base oil No. 7 per acre. Cucumber downy mildew infection was decreased 25 per cent by fungicide-oil mist treatment.

Susceptibility to common blight of bean as influenced by level of nutrition. E. M. STODDARD. The effect of the nutrition level on the susceptibility of Red Kidney bean to common blight (*Xanthomonas phascoli*) was studied on plants grown in sand at nutrient level increments of 1, 10, and 100. The nutrient solution used contained potassium nitrate, monopotassium phosphate, calcium nitrate and magnesium sulfate. Inoculation was accomplished by stabbing the causal organism into the stem. The data were recorded 13 days after inoculation. The percentages of plants showing wilting of the foliage at the above ascending order of nutrient levels were 91, 88, and 21. The inoculated plants at the highest nutrient level showed more stunting than either those at the lower levels or the checks. The causal organism was isolated from the plants not showing wilting at the highest level, indicating that these plants tolerated the organism without exhibiting the usual wilting symptoms.

REPORT OF THE 39TH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The American Phytopathological Society held its 39th annual meeting at the Stevens Hotel, Chicago, Illinois, December 28–30, 1947, in connection with the American Association for the Advancement of Science. Three hundred and thirteen members from forty-two states and three foreign countries registered. One hundred and twenty-seven papers, accepted by the editorial committee, were presented at the meeting. The sections and number of papers presented in each follow: diseases of corn and small grains, 13; diseases of fruits and ornamentals, 13; physiology of fungi, 12; diseases of field crops, 10; virus diseases, 11; breeding and disease resistance, 10; joint session with the Potato Association of America, 5; antibiotics and bacterial diseases, 13; virus diseases of vegetables, 6; fungicides, 10; vegetable diseases, 11; and miscellaneous, 13.

A joint symposium was held with the Committee on Genetics of Microorganisms of the National Research Council, the American Society of Naturalists, the Mycological Society of America, and the Microbiological Section of the Botanical Society of America. A joint session was also held with the Potato Association of America.

Conferences included "Fungicide Colloquium," "Extension Workers," "Plant Disease Survey," "National Security," and "Upper Mississippi Valley Plant Pathologists."

There were demonstration sections on Market Diseases of Fruits and Vegetables, and Publicity.

The premiere showing of the color, sound, moving picture "White Pine Blister Rust Control" was presented by the United States Department of Agriculture.

The Phytopathologists' dinner, held at the Chicago Bar Association, on Monday evening, December 29, was attended by over three hundred.

Council for 1948:

R. S. KIRBY, President (1 yr.), Pennsylvania State College, State College, Pennsylvania.
W. D. VALLEAU, Vice-President (1 yr.), Kentucky Agricultural Experiment Station, Lexington 29, Kentucky.
CURTIS, MAY, Secretary (3-yr. term expires 1950), Plant Industry Station, Beltsville, Maryland.
M. C. RICHARDS, Treasurer, and Business Manager of PHYTOPATHOLOGY (3 yr. term expires 1949), University of New Hampshire, Durham, New Hampshire.
HELEN HART, Editor-in-Chief, PHYTOPATHOLOGY (3-yr. term expires 1948), University Farm, St. Paul 1, Minnesota.
E. E. CLAYTON, Plant Industry Station, Beltsville, Maryland.
MAX W. GARDNER, University of California, Berkeley, California.
J. H. JENSEN, North Carolina State College, Raleigh, North Carolina.
S. G. LEHMAN, North Carolina State College, Raleigh, North Carolina.
S. E. A. MCCALLAN, Boyce Thompson Institute, Yonkers 3, New York.
S. J. P. CHILTON, Louisiana State University, Baton Rouge, Louisiana.
A. J. RIKER, University of Wisconsin, Madison, Wisconsin.

Representatives:

A.A.A.S. Council. R. W. GOSS, H. A. RODENHISER.
Division of Biology and Agriculture, National Research Council. J. C. WALKER.
Board of Editors, American Journal of Botany. CHARLES CHUPP.
American Institute of Biological Sciences. J. C. WALKER.

Standing Committees:

Donations and Legacies. H. S. FAWCETT, P. P. PIRONE, R. E. VAUGHN, A. A. DUNLAP, W. B. TISDALE, N. J. GIDDINGS, Chm.
Extension. O. C. BOYD, O. D. BURKE, L. O. WEAVER, R. J. HASKELL, J. O. ANDES, S. B. FENNE, JOHN R. VAUGHN, W. J. HENDERSON, E. G. SHARVELLE, J. T. MIDDLETON, T. H. KING, Chm.
Investments. MARVIN E. FOWLER, E. L. LECLERG, NEIL E. STEVENS, R. M. CALDWELL, M. C. RICHARDS, Chm.

Phytopathological Classics. L. J. TYLER, Business Manager; L. C. KNORR, Editor.
Placement. H. A. RODENHISER, M. W. GARDNER, R. J. HASKELL, L. M. MASSEY, S. J. P. CHILTON, R. S. KIRBY, Chm.
Public Relations. O. D. BURKE, L. S. HITCHNER, J. D. MOORE, A. G. NEWHALL, HARRY R. O'BRIEN, F. J. GREANEY, H. T. COOK, J. E. LIVINGSTON, E. C. COX, R. R. KINCAID, K. STARR CHESTER, Chm.
International Cooperation. G. H. COONS, K. G. KEVORKIAN, I. E. MELHUS, M. H. LANGFORD, J. G. HARRAR, J. A. STEVENSON, OTTO REINKING, H. P. BARSS, E. C. STAKMAN, Chm.
Society Organization. W. J. ZAUMEYER, A. L. HARRISON, L. W. KOCH, J C. WALKER, O. C. BOYD, GEORGE ARMSTRONG, Chm.
Plant Disease Prevention. PAUL R. MILLER, S. B. FRACKER, E. C. STAKMAN, H. H. THORNBERRY, D. G. FLETCHER, J. J. CHRISTENSEN, Chm.
Subcommittee on Regulatory Work and Foreign Plant Diseases. C. R. ORTON, R. P. WHITE, E. C. STAKMAN, Chm.
Subcommittee on Seed and Plant Material Certification. REINER BONDE, L. C. COCHRAN, CHARLES CHUPP, C. H. ARNDT, J. J. CHRISTENSEN, Chm.
Subcommittee on Utilization of Plant Pathologists and Field Facilities in National Emergencies. M. W. GARDNER, LESLIE WALDEE, H. T. COOK, H. H. THORNBERRY, Chm.

Special Committees:

Fungicides. C. H. ARNDT, J. D. WILSON, J. W. HEUBERGER, F. L. HOWARD, DWIGHT POWELL, C. E. YARWOOD, J. G. HORSFALL, Chm.
Subcommittee on Seed Treatments. E. D. HANSING, H. T. COOK, GEORGE SEMENIUK, J. E. MACHACEK, C. H. ARNDT, Chm.
Subcommittee on Dusts and Sprays. J. M. HAMILTON, A. W. DIMOCK, A. G. NEWHALL, J. W. HEUBERGER, W. F. BUCHHOLTZ, H. F. WINTER, M. B. LINN, J. D. WILSON, Chm.
Subcommittee on Special Problems. L. S. HITCHNER, E. A. WALKER, J. G. LEACH, F. L. HOWARD, Chm.
Subcommittee on Annual Summary of Results on Newer Fungicides. PAUL R. MILLER, E. E. CLAYTON, M. C. GOLDSWORTHY, R. W. LEUKEL, R. J. HASKELL, W. D. MCCLELLAN, J. W. HEUBERGER, Chm.
Subcommittee on Fungicide Nomenclature. J. D. MOORE, W. H. TISDALE, J. C. DUNEGAN, S. L. HOPPERSTEAD, E. G. SHARVELLE, DWIGHT POWELL, Chm.
Fungus Nomenclature. W. W. RAY, W. C. SNYDER, D. E. ELLIS, C. M. CHRISTENSEN, J. A. STEVENSON, Chm.
Nomenclature and Classification of Plant Viruses. FREEMAN WEISS, C. W. BENNETT, H. H. MCKINNEY, JAMES JOHNSON, R. H. LARSON, H. R. MCLARTY, FRANK MCWHORTER, L. M. BLACK, Chm.
Subcommittee on Virus Type Culture Collection. JAMES JOHNSON, THEODORE J. GRANT, H. H. MCKINNEY, Chm.
Membership. W. J. JEFFERS, T. W. BRETZ, B. H. DAVIS, C. M. CLAYTON, THOR KOMMEDAHL, GLENN S. POUND, C. M. TOMPKINS, Chm.
Sustaining Associates. J. D. WILSON, E. G. REX, P. D. PETERSON, E. G. SHARVELLE, FRED LEWIS, P. P. PIRONE, Chm.
Nomenclature of Plant Pathogenic Bacteria. J. G. LEACH, W. H. BURKHOLDER, CHARLOTTE ELLIOTT, E. M. HILDEBRAND, P. A. ARK, O. N. ALLEN, Chm.
Teaching of Plant Pathology. J. G. LEACH, G. C. KENT, C. M. TUCKER, M. F. KERNKAMP, G. F. WEBER, Chm.

Temporary Committees for 1948:

Membership List Committee. HELEN HART, M. C. RICHARDS, L. J. ALEXANDER, Chm.
Publication of Special Material. H. P. BARSS, J. G. LEACH, W. H. TISDALE, DONALD CATION, Chm.

Report of the Secretary. On November 5, 1947, the total membership was 1322, consisting of 145 life members, 1096 regular members, and 81 applicants to be inducted at the Chicago meeting of the Society. During the year, 45 members were suspended for nonpayment of dues, 3 died, and 12 resigned. Twenty-seven additional applications were received after November 5. The membership as of December 31, 1947 is about 1350.

Report of the Treasurer. Statement of accounts for the year ending September 30, 1947.

Receipts:

Balance from 1946			$ 2,781.58
Annual dues:			
1946	$ 20.00		
1947	5,683.49		
1948	2,112.16		
1949	8.00		
1950	.50	$7,824.15	
30-Year Index		6.00	
Sales		68.00	
Membership List		.50	
1947 Subscription		6.50	
Surplus from 12/46 banquet		17.00	
Sustaining Associates		1,900.00	
Total receipts			9,822.15
			$12,603.73

Expenditures:

Member subscriptions transferred to PHYTOPATHOLOGY			
1946	$ 16.00		
1947	4,537.64		
1948	1,759.23		
1949	6.62		
1950	.42	$6,319.91	
Transferred to PHYTOPATHOLOGY for:			
30-Year Index	6.00		
Sales	68.00		
Membership list	.50		
1947 subscription	6.50	81.00	
Office of Secretary			
Secretarial work	46.20		
Stamps	52.86		
Printing	245.14		
Other	13.21	357.41	
Office of Treasurer			
Secretarial work	540.80		
Supplies	73.17		
Express	94.11		
Bank charges	26.30		
Auditing	25.00		
Printing	4.24		
Other expenses	18.73	782.35	
Biological Abstracts	100.00		
Placement Committee	16.60		
Sustaining Associates Committee	10.00		
Total expenditures			7,667.27
Balance on hand September 30, 1947			4,936.46
			$12,603.73

Report of the Business Manager. During the past year the costs for printing PHYTOPATHOLOGY and reprints from PHYTOPATHOLOGY increased another 10 per cent, making a total over pre-war prices of 30 per cent for PHYTOPATHOLOGY and 20 per cent for reprints. Certain functions formerly done by the printer, such as typing of mailing slips and the billing of authors for reprints, were transferred to the Business Office.

Total non-member subscriptions on November 10, 1947, were 840, and adding to these the 1320 member subscriptions gives a total distribution for PHYTOPATHOLOGY of 2,160.

The sales of back volumes during 1947 continued high, amounting to $2,032.27.

Statement of accounts for the year ending September 30, 1947.

Receipts:			
Balance from 1946			$ 9,980.81
Subscriptions:			
1946	$ 25.10		
1947	4,595.40		
1948	511.60		
1949	39.50	$5,171.60	
Member subscriptions:			
1946	16.00		
1947	4,537.64		
1948	1,759.23		
1949	6.62		
1950	.42	6,319.91	
Sales of back numbers of PHYTOPATHOLOGY		2,032.27	
Sales of Membership List		4.50	
Advertising			
1946	379.18		
1947	885.34	1,264.52	
30-Year Index		112.55	
Interest on current funds			
First mortgage	20.25		
Building and Loan	50.00		
U. S. Bond, Series G.	25.00	95.25	
Interest on current funds		127.51	
Grant from Rockefeller Institute		600.00	
Allowance on reprints		825.64	
From authors for excess illustrations		318.05	
Unused credit		38.90	
Membership dues		26.00	
Sustaining Associates		200.00	
Total receipts			17,136.70
			$27,117.51
Expenditures:			
Printing, distributing, and storing PHYTOPATHOLOGY:			
Vol. 36, no. 9	$740.82		
10	887.00		
11	768.79		
12	958.71		
Vol. 37, no. 1	703.68		
2	830.72		
3	631.43		
4	752.17		
5	987.52		
6	701.19		
7	861.72		
8	775.60		
Engravings, Sept. '46 to Aug. '47	944.76	$10,544.11	
Postage, PHYTOPATHOLOGY		929.53	$11,473.64
Secretarial work and office expense, Editor-in-Chief			1,253.34
do Advertising Mgr.			47.65
Commission for Advertising Manager, 1946			92.50
Office of the Business Manager			
Secretarial work			546.70
Office supplies			38.14
Stamps			180.47
Miscellaneous			69.66
Refund, subscriptions and sales			307.41
Purchase, back volumes			215.50
Express			33.97
Printing			119.31
Binding volumes			15.39
Transfer, APS dues			45.50
Transfer, APS Sustaining Assoc. contributions			200.00
Miscellaneous			21.73
Total expenditures			$14,660.91
Balance on hand:			
Checking account		7,259.93	
Northwestern Federal Savings & Loan (Wash., D. C.)		5,196.67	12,456.60
			$27,117.51

Sinking Fund. By the completion of payment on a life membership by an Italian member, the principle amount of the sinking fund was increased this year by $30.34, it now being $9,706.34.

First mortgage note, at 4¼ per cent interest, deposited with McLachlen Banking Corporation for collection	$ 500.00
U. S. Savings Bond, Series G, No. M19056020, 2½ per cent	1,000.00
Invested with the following:	
Jefferson Federal Savings (formerly Columbia Permanent Bldg. Assoc.) (accrued dividends $85.96)	585.96
District Bldg. Loan Association (accrued dividends $253.67)	1,753.67
National Permanent Bldg. Assoc. (accrued dividends $390.03)	2,390.03
Northwestern Fed. Savings & Loan Assoc. (certificates)	2,000.00
Perpetual Building Association (accrued dividends $171.92)	1,171.92
Prudential Building Association (accrued dividends $38.69)	245.03
Arlington and Fairfax Bldg. & Loan (accrued dividends $77.26)	1,077.26
	$10,723.87
Less interest due PHYTOPATHOLOGY	1,017.53
	$ 9,706.34

The Lyman Memorial Fund, obtained from voluntary contributions, now totals $3,321.82. This amount is invested with the Brookland Building and Loan Association at 2½ per cent. The account for 1947 is as follows:

Balance on hand, October 1, 1946	$ 3,703.80
Dividends, December 31, 1946 and June 30, 1947	93.16
Voluntary contributions	11.00
	$ 3,807.96
Less interest due PHYTOPATHOLOGY	486.14
	$ 3,321.82

Additional Endowment:

War Savings Bond, Series F	
Total, September 30, 1947	$1,125.00
War Savings Stamps	
Total, September 30, 1947	7.00
	$1,132.00

The 30-Year Index. Summary of receipts (funds deposited in PHYTOPATHOLOGY checking account) October 1, 1946 to September 30, 1947:

Balance (receipts less costs) September 30, 1946	$373.52
Receipts October 1, 1946 to September 30, 1947	112.55
Balance on hand, September 30, 1947	$486.07
Membership List Account, 1946–1947	
Balance on hand, October 1, 1946	$ 52.46
Membership lists sold	4.50
Balance on hand, September 30, 1947	$ 56.96

Report of the Auditing Committee, as of September 30, 1947. We have examined the books of the Treasurer of the American Phytopathological Society and of the Business Manager of PHYTOPATHOLOGY for the period from January 22, 1947 to September 30, 1947. We found all receipts and expenditures and all funds of the Society and of PHYTOPATHOLOGY lucidly and accurately recorded.

Signed: STUART DUNN, *Chairman*
M. D. FARRAR

Report of the Advertising Manager. PHYTOPATHOLOGY carried a total of 94 pages of paid advertisements in 1947. The gross income from advertising was $1625.60. The net income to the Society was $1382.67. The sixteen companies which advertised in 1947 have been written concerning renewal of contracts for 1948. Six companies have contracted for 27½ pages for a total of $1260. Two other companies are likely to contract for 24 for a total of $928.80. Three companies have canceled

their contracts, and five have not replied. The slowness in new contracts is due to increased rates and to the fact that some contracts do not expire until early 1948. It now appears that there will be fewer advertisers in 1948, but the income will possibly be more than in 1947.

Report of the Editor-in-Chief. Volume 37 of PHYTOPATHOLOGY contained only 931 pages, but 335 authors used these pages in the publication of 89 long articles, 37 phytopathological notes, 209 abstracts, 2 biographies, 2 committee reports, and 8 book reviews. The report of the annual meeting, the revised constitution, and 3 announcements were published for the Society.

The volume contains 2 portraits, 151 illustrations for scientific articles and notes, and 251 tables. Authors have been charged for illustrations exceeding 2 pages and for tables exceeding 33 per cent of the length of a paper. Divisions have been charged for the divisional abstracts of members for whom the Society published two abstracts presented at the annual meeting.

On December 1, 1947, there were 64 papers on hand; 35 had been accepted for publication and 28 of these were in press; 13 were being revised by the authors; and 16 were being reviewed by the editors. Between December 1, 1946 and December 1, 1947, eleven papers were withdrawn or rejected.

Transference of responsibility for reprint orders from The Science Press to The American Phytopathological Society delayed slightly the publication of some papers that might otherwise have been scheduled for the November and December issues. The necessity for price quotations on special forms and delay of the issuance of requisitions and purchase orders may account for a delay in publication of an article.

Report of Representative on Division of Biology and Agriculture, National Research Council, 1947. The activity of the Division of particular concern to our Society at the annual meeting in April, 1946 had to do with the proposed American Institute of Biological Societies. The proposed Constitution and By-Laws passed by the Organizing Committee, called together by the Division, were discussed and approved for reference back to the individual societies for consideration. Copies of the Constitution and By-Laws were distributed to members of the Society previous to the Chicago meeting. A discussion of these documents was submitted by your representative to the Council.

Report of the Necrology Committee. The following members died during 1947: A. E. DAVEY, ALBERT ISAKSSON, and RALPH JUSTO-PRATS.

Report of the Manager of Phytopathological Classics. Report for the fiscal year beginning October 1, 1946 and ending September 30, 1947:

Classic No. 1:	On hand, October 1, 1946	0	
Classic No. 2:	On hand, October 1, 1946	178	
	Sold during year	12	$ 6.00
	On hand, September 30, 1947	166	
Classic No. 3:	On hand, October 1, 1946	265	
	Sold during year	12	6.00
	On hand, September 30, 1947	253	
Classic No. 4:	On hand, October 1, 1946	328	
	Sold during year	11	8.25
	On hand, September 30, 1947	317	
Classic No. 5:	On hand, October 1, 1946	561	
	Sold during year	14	17.50
	On hand, September 30, 1947	547	
Classic No. 6:	On hand, October 1, 1946	646	
	Sold during year	13	9.75
	On hand, September 30, 1947	633	
Classic No. 7:	On hand, October 1, 1946	664	
	Sold during year	13	9.75
	On hand, September 30, 1947	651	
			$ 57.25

Value of books sent out (fiscal year 1946–1947)	$ 57.25
Money received as advance on Classic No. 8	1.00
Money received on orders of previous year	7.61
	$ 65.86
Money received during fiscal year 1946–1947	$ 61.36
Due on account	4.50
	$ 65.86

Assets:	
Cash balance on hand, October 1, 1946	$642.28
Receipts during year	61.36
	$703.64
Liabilities:	
Stamps	$ 5.00
Express charges	3.39
	$ 8.39
Balance on hand, September 30, 1947	$695.25
Total due on account, September 30, 1947	$ 9.75

Report of the Editor of Phytopathological Classics. Number 8 of PHYTOPATHOLOGICAL CLASSICS will be off the press by year's end. Final page proofs are now in process of correction. Number 8, containing M. J. Berkeley's "Observations, Botanical and Physiological on the Potato Murrain" and selections from his "Vegetable Pathology," was prepared by a committee of the British Mycological Society, with Dr. John Ramsbottom contributing a 3500-word illustrated biography. The price of the 100-odd-page publication has been set at $1.25. All expenses incident to the new volume will be paid out of proceeds from the sale of previous issues, thereby continuing the policy of keeping PHYTOPATHOLOGICAL CLASSICS on a self-sufficient basis.

Editing of the Berkeley manuscript entailed much labor, especially in comparing the galley proofs with the originals. Petition for funds to engage an assistant was made to Michigan State College's All-College Research Fund in the amount of $30.00, and the petition was generously granted to the extent of $50.00. I suggest the Council write Professor L. C. Emmons, Dean of the School of Science and Arts, Michigan State College, East Lansing, Michigan, thanking him for his influence in arranging the grant. Copy of such action to me would also be appreciated.

Plans for future issues of PHYTOPATHOLOGICAL CLASSICS are under way, and Tozetti's ALIMURGIA appears to be a likely subject for CLASSIC NO. 9. Tozetti's writings have already been reviewed by Doctors P. P. Pirone and J. C. Walker, both of whom deem the selection an interesting and worth-while treatise for inclusion in the series.

In discussing future plans, however, there is one proviso I should like to suggest, and that is that any subsequent publishing be postponed until printing costs have stopped spiralling. The per unit cost of PHYTOPATHOLOGICAL CLASSICS would soon preclude sales, and I am not willing to see the 15-year-old record of solvency lost.

Report of the Placement Committee. During 1947, 54 plant pathologists had applications filed with the Placement Committee. Prospective employers listed 63 positions with the Committee. A total of 214 individual applications were sent to employers. Incomplete reports indicate that at least 5 plant pathologists obtained positions through the efforts of this Committee.

Report of the Public Relations Committee for 1947. The procedure in publicizing plant disease research has continued as in the past with the exception that "IDEAS" for popular articles have usually been routed directly to editors of the most suitable journals instead of using a wider, impersonal distribution. Nineteen "IDEAS" were released, of which 16 resulted in published articles in "Farm Journal," "Country Gentleman," "Better Homes and Gardens," "Scientific Monthly," "Farmer-Stockman," and other periodicals. In several instances the Committee has been of service to the fungicide industry in providing information on preventable crop losses and markets for fungicides. The Chairman of the Committee has been appointed to represent the Society in providing editorial assistance for "Scientific Monthly." An exhibit of the Committee's work was on display at the Chicago meeting. The expenses of the Committee, as in the past, have been paid from remuneration received by individual writers for magazine contributions.

Report of the Committee on Biological Abstracts and Union of Biological Societies. The coverage of biological journals by Biological Abstracts has been rapidly increased during 1946 and 1947 to include as many as possible of the journals which could not be covered during the war and new journals published in Europe and Asia. Up to July 1, 1947, over 850 assignments covering these journals had been made in the preceding 18 months. The total journals being abstracted now number about 2800. Because of the continued unavailability of many foreign publications, the abstracting of a large share of these journals is being carried out by collaborators in the country

of origin of the journal. The Japanese journals are the only ones still unavailable for abstracting.

The increased cost of printing and binding has put a serious strain on the finances of Biological Abstracts. In 1946 it was necessary to withdraw $1320 from reserves. In 1947, with no visible large increase in revenues, the printing and mailing estimates alone have been increased $13,000 over 1946. A concerted effort for further support will be necessary.

The situation is more serious since the recent delays in appearance of the numbers, due entirely to difficulties in the printing and binding of the material, do not make good advertising. Abstracting is being kept up-to-date.

During 1946 Section H—Abstracts of Human Biology—was added. Beginning with January 1948 a new section—Abstracts of Cereals and Cereal Products—will be added. The latter will contain abstracts otherwise scattered through other sections of Biological Abstracts.

Report of the Committee on Regulatory Work and Foreign Plant Diseases. This Committee has been relatively inactive. It was appointed at the suggestion of Dr. P. N. Annand, Chief of the Bureau of Entomology and Plant Quarantine, as a committee to give advice on request. Only one question has been submitted, and I hope that this was satisfactorily answered. The recommendation is that the Committee be continued and that the chairman ascertain its possible future need and usefulness.

Report of the Committee on International Cooperation. Several members of the Committee met in Washington and discussed policy and activities. Various members of the Committee also discussed matters with officials of the Office of Foreign Agricultural Relations. The chairman of the Committee, on an extensive trip through Mexico, Central and South America, discussed with many pathologists the question of interchange of information such as that previously proposed by the Committee. There was enthusiastic response with respect to the desirability of this interchange, but it was evident that it was impossible to get the desired information from many countries because of lack of pathologists, lack of organization, or lack of facilities for accumulating and summarizing the necessary information. This makes it seem possible that it would be better to start with a less ambitious program.

A modified proposal therefore is made with respect to the interchange of information.

It is proposed that the Committee on International Cooperation of the American Phytopathological Society collaborate with the Committee on Inter-American Scientific Publication (Harlow Shapley, chairman; Mrs. Christina Buechner, executive secretary). Steps already have been taken to have certain summaries regarding plant diseases published in Latin American journals. The objective of the Committee on I.A.S.P. at present is to facilitate the interchange of scientific information between countries of the Western Hemisphere. It is hoped, however, to expand the project into other countries as soon as improvements in the international situation warrant it. As this organization has a central office in Boston, with a full-time secretary, and is desirous of facilitating publication of scientific papers in general; and as they have at least a modest sum of money as a working fund, it is proposed that we collaborate with them as a first step in attaining the broader objectives envisaged by the Committee.

It is recommended that the Committee be continued.

Report of the Committee on Resolutions. Be it resolved that the American Phytopathological Society express its grateful appreciation to the following for their contributions to the success of the 39th annual meeting:

W. B. Allington, J. C. Carter, Glenn S. Pound, and M. A. Smith for so capably handling all arrangements pertaining to the banquet, entertainment, and smooth functioning of all sessions.

A. C. Hildebrand, J. E. Kuntz, and John E. Thomas for supervising registration.

W. D. Valleau and Stephen Diachun for arranging all papers into sessions.

K. Starr Chester for so ably handling all publicity before and during the meeting.

The management of Hotel Stevens, and especially John W. Bowman, Director of Conventions and Exhibits, for many courtesies extended the Society.

The Chicago Bar Association for splendid cooperation in providing banquet facilities.

John Strohm, Associate Editor of "Country Gentleman," for his most interesting and informative address on Russian agriculture.

The American Association for the Advancement of Science for providing meeting rooms, projection equipment, operators; and other courtesies.

Be it separately resolved that the Society express its appreciation to President A. J. Riker, Vice-President R. S. Kirby, and Secretary E. M. Johnson for invaluable services during their terms of office, and to other officers, representatives, councilors, and committee members for their efficient work in conducting various activities of the Society during the past year.

Elections and Appointments. A committee from the Council opened and counted the ballots, results of which were announced to the Society at the banquet the evening of December 29: R. S. Kirby, President; W. D. Valleau, Vice-President; S. J. P. Chilton, Councilor-at-large. Curtis May was appointed Secretary for 1948–1950.

The Council recommended and the Society approved the appointment of J. H. Jensen as Editor for a three-year term through 1950; J. D. Wilson, J. G. Harrar, K. W. Kreitlow and Donald Cation as Associate Editors for three years through 1950; L. J. Alexander, Advertising Manager of PHYTOPATHOLOGY for 1948; L. C. Knorr, Editor of PHYTOPATHOLOGICAL CLASSICS for 1948; L. J. Tyler, Business Manager of PHYTOPATHOLOGICAL CLASSICS for 1948; J. C. Walker, Representative to American Institute of Biological Science; H. A. Rodenhiser on A.A.A.S. Council for 1948–49; and Charles Chupp on the Board of Editors, American Journal of Botany for 1948–50.

One hundred and eight applicants were elected to membership in The American Phytopathological Society.

Reports of Officers, Representatives, and Standing Committees are published on the previous pages. According to Society action at the Philadelphia meeting, reports of Special and Temporary Committees are not to be published in the annual report. All Committee reports submitted were considered by the Council. Those recommended for approval by the Council were accepted by the Society.

The Society approved the following recommendations by the Council:

1. That the Membership List Committee be given permission to publish a membership list in 1948.
2. That the Special Committee on Plant Disease Prevention be made a standing committee.
3. That the Standing Committee on Regulatory Work and Foreign Plant Diseases be made a subcommittee of the Plant Disease Prevention Committee.
4. That the name of the Special Committee on National Security be changed to Subcommittee on the Utilization of Plant Pathologists and Facilities in National Emergencies, and that it be under the Plant Disease Prevention Committee.
5. That there be added to the Plant Disease Prevention Committee a Subcommittee on Seed and Plant Material Certification Standards.
6. That the Standing Committee on Necrology be discontinued and that its functions, including the preparation of biographical sketches of deceased members, be assigned to the Secretary.
7. That the Standing Committee on the Union of Biological Societies (Biological Abstracts) be discontinued and that its functions be taken over by the Society's representative to the American Institute of Biological Sciences.
8. That a Subcommittee on Virus Type Culture Collection be added to the Committee on Nomenclature and Classification of Plant Viruses.
9. That the Temporary Committee on Sustaining Associates be made a special committee.
10. That the Fungicide Committee consist of seven members.
11. That the Subcommittee on Methods of the Fungicide Committee be discontinued for the time being.
12. That there be added to the Fungicide Committee a Subcommittee on Annual Summary of Results on Newer Fungicides, to continue the work initiated by the Potomac Division in disseminating this information.
13. That there be added to the Fungicide Committee a Subcommittee on Fungicide Nomenclature whose function shall be to study the important question of common names of fungicides in cooperation with other interested committees from the chemical, entomological, and other societies and the United States Department of Agriculture, and that they be further authorized to select suitable common names and to preempt their use by such means as they deem advisable, and that such names shall be published in PHYTOPATHOLOGY when they are ready for adoption.
14. That there be formed a Special Committee on Nomenclature of Plant Pathogenic Bacteria.
15. That there be formed a Special Committee on Teaching of Plant Pathology.
16. That the invitation to join the American Institute of Biological Sciences be submitted to the members for a mail vote, in a reasonable time, and if approved the dues will be increased one dollar beginning in 1949.
17. That the Society continue to appropriate $100 to Biological Abstracts.
18. The adoption of the following resolution: Be it resolved that The American Phytopathological Society express its interest in the proposal of Dr. Paul Neergaard for the issuance of an International Herbarium of Pathogenic Fungi (fungi exsiccati) to be

initiated and carried on under the auspices of U.N.E.S.C.O. or similar international organization, and agree to join with the Mycological Society of America and the British Mycological Society in sponsoring the project. Suitable precautions should be taken to guard against dissemination of potentially dangerous parasites. It is understood no financial outlay is involved.

19. That the editors of PHYTOPATHOLOGY are authorized to publish sheets containing news, notes, announcements, comments, and short items of interest. The activity is not to cost over $300 in 1948.

20. The adoption of the following resolution:

Whereas many foreign plant pathogens not only have already come into the United States but also have caused enormous damage, and

Whereas there are many other plant pathogens which might cause similar devastating loss if they should enter and become established, The American Phytopathological Society resolves that:

(A) The United States needs to have a greatly strengthened plant disease quarantine service at ports of entry or in countries of origin. Many plants carry diseases which do not show on seed or dormant stock. Such material should be given competent inspection while growing. This inspection should meet the standards now applied to nursery stock in most states. This is particularly important since pathogens brought in by aeroplanes will arrive more promptly and usually in more virulent condition than organisms introduced by the older forms of transportation.

(B) The United States needs more critical information about the susceptibility of American timber species and crop plant varieties to foreign parasites that might be introduced.

(C) More effectively to meet these needs, it is recommended that the U. S. Department of Agriculture request the necessary additional appropriations and arrange with the Department of State for development of cooperative agreements with those foreign countries interested in safe plant exchange on a mutually beneficial basis. The activity should include:

(a) the establishment in the cooperating country of test or trap plantings of important American species and varieties of interest to either cooperating country, for which the reaction to foreign pests and diseases needs to be ascertained;

(b) periodic observation by competent American and/or foreign specialists for diseases; and

(c) for those found important, study of the means of dissemination of the causal organisms.

(D) Similarly, test planting of important species and varieties of the cooperating countries may be established in appropriate regions of the United States.

21. The formation of the North Central Division of The American Phytopathological Society, an area including the states of Missouri, Minnesota, Kansas, Nebraska, South Dakota, North Dakota, Iowa, Illinois, Kentucky, Wisconsin, Michigan, Ohio, and Indiana.

22. That the time and place of the next annual meeting shall be left to the discretion of the Program Committee consisting of the President, Vice-President, Secretary, and Editor-in-Chief, with the suggestion that the meeting be held in early December, in connection with other societies, in the longitude of Pittsburgh or farther east.

23. That the Special Committee on Publication Problems be discontinued.

24. That there be published in 1948, in PHYTOPATHOLOGY, a Directory of Fungicides consisting of a list of fungicides with chemical, trade, and common names (if available) with the name of the manufacturer, as shall be prepared by the Subcommittee on Fungicide Nomenclature.

GROVER H. BURNETT

January 31, 1890–June 15, 1946

Dr. Grover H. Burnett, a native of Idaho, was graduated from the University of Idaho, 1917, with the degree of Bachelor of Science. Entering World War I as a Private, he earned a Commission. He then spent eight years in educational work in agriculture in Idaho, Washington, and California. From 1927 to 1933 he was on the staff of the Botany and Plant Pathology Department of Washington State College, where he received the degree of Master of Science in 1929 and Doctor of Philosophy in 1932.

The years 1933 to 1936 he spent in white pine blister rust control in the Northwest, and in study of the nursery diseases of conifers in Montana, Colorado, and Nebraska, for the Bureaus of Entomology and Plant Quarantine, and Plant Industry, respectively. From that time until his death he was engaged as Farm Planner for the Soil Conservation Service in Colorado.

Doctor Burnett was a man of good judgment and pleasing personality, an excellent cooperator and universally liked. During his period at Washington State College he published a number of creditable papers on the viruses of the Solanaceae and of Phlox and Delphinium. He took particular pleasure in the out-of-door features of his work. He was cautious in interpretation of results, and outstanding for the conscientious character of his work.

Doctor Burnett was a life member of The American Phytopathological Society.

JOSEPH HUNTER GOODING, JR.

August 31, 1891–April 14, 1947

J. Hunter Gooding, Jr., was graduated from Oregon State College in 1914 with the Bachelor of Science degree in Agronomy. He took advance courses at Columbia University, New York; New York University, and the University of Pennsylvania.

Except for two intervals as farm manager and orchardist, Mr. Gooding's entire career was with E. I. du Pont de Nemours and Company. In his 32 years with the company he rose from a clerkship at the Carney's Point, New Jersey plant to sales manager of the Semesan Division of the Grasselli Chemicals Department.

It was in 1923 that Mr. Gooding began his career in the field of seed disinfectants. By the time of his death he was recognized as one of the men who had made valuable contributions toward establishing the need for seed disinfection as common practice in the minds of the American farmer.

Mr. Gooding was a member of the American Phytopathological Society, The Grange, and other organizations.

Mr. Gooding has been described as being square in his dealings with coworkers and competitors alike, competent, cheerful, optimistic, and sociable. He was a fine example of the kind of person it takes for successful working relations between science and industry.

ROBERT ALMER HARPER

January 21, 1862–May 12, 1946

Robert Almer Harper was born at Le Claire, Iowa. He graduated from Oberlin College with the degree of Bachelor of Arts in 1886 and carried on graduate studies at Johns Hopkins University during 1888–89. He returned to Oberlin to receive the degree of Master of Arts in 1891. Following this he studied for several years at Bonn under the great botanist, Eduard Strasburger, receiving the doctorate from the University of Bonn in 1896.

Professor Harper's long and distinguished career as a teacher began when he went to Gates College as instructor of classical languages from 1886 to 1888. From 1889 to 1898 he was instructor and professor of botany and geology at Lake Forest College, Lake Forest, Illinois. From 1898 to 1911 he was professor and head of the Department of Botany at the University of Wisconsin. In 1911 Dr. Harper went to Columbia University as Torrey Professor of Botany and executive officer of the department. He withdrew from formal teaching in 1930 with the title of Emeritus Professor, but continued active in research and advisory work with graduate students at Columbia until 1937, when he moved to his farm near Bedford, Virginia, where his remaining years were spent.

Dr. Harper was active in the National Academy of Sciences, American Association for the Advancement of Science (vice-president for the plant sciences section in 1910), Botanical Society of America (president in 1916), Torrey Botanical Club (president 1914–16), American Phytopathological Society, Ecological Society of America, Society of American Naturalists, Philosophical Society, American Academy, and was a corresponding member of the Deutsche Botanische Gesellschaft.

In Professor Harper the qualities of a great, inspiring teacher and a penetrating, painstaking research worker were perfectly combined. His earlier works on the cytological details of reproduction in the powdery mildews, in Peziza, Pyronema, Hypochnus, and several rusts have long been considered classics in the field. Later his interest turned to problems of the fundamental structure and morphogenesis of living things. His presidential address before the Botanical Society in 1916 was an exceedingly penetrating consideration of the structure of protoplasm. His later papers deal with the factors involved in the development of form. Professor Harper's grasp of the structure and interrelationships of living things and his insight into fundamental biological problems were unparalleled.

JOHN LEONARD RUE

July 15, 1900–February 9, 1946

John Leonard Rue was educated in the public schools at Freehold, New Jersey, after which he studied at Stevens Institute of Technology, Hoboken, New Jersey.

Mr. Rue was employed by the Bureau of Plant Industry, U. S. Department of Agriculture, in 1927, to assist at the Bureau's Peach Disease Field Laboratory, Fort Valley, Georgia. He was appointed Scientific Aid in 1931, advanced through grades, and was promoted to Chief Scientific Aid in 1941.

During his employment at Fort Valley, he assisted in planning laboratory buildings and structures, maintained experimental nurseries, orchards, and greenhouses, and participated in investigations on the phony peach disease, peach rosette, winter injury, prolonged dormancy of the peach, and other research activities. In 1938 he was transferred to a new laboratory being established at Brownwood, Texas, by the Division of Fruit and Vegetable Crops and Diseases, for investigations on peach mosaic and other virus diseases of deciduous fruit trees, in which work he remained until his death.

Mr. Rue was a most loyal and dependable worker. He developed investigative ability, became proficient in virus research techniques, and was coauthor of several technical publications. His straightforward character and excellent personality endeared him to his associates and greatly furthered the cooperative work in which he was engaged.

GORDON ALEXANDER SCOTT

May 7, 1895–February 28, 1946

Gordon Alexander Scott, after four years' military service in World War I, graduated from the Ontario Agricultural College, Guelph (University of Toronto) in 1923 with the degree of Bachelor of Science in Agriculture. He received the degree of Master of Science from Macdonald College, St. Anne de Bellevue, Quebec (McGill University), in 1924 where he held the W. C. Macdonald scholarship in 1923 and the Chilean Nitrate scholarship in 1924.

In February, 1925, Mr. Scott was appointed to the Division of Botany and Plant Pathology and was stationed at the Dominion Laboratory of Plant Pathology, Saskatoon, Sask., under the late Professor W. P. Fraser. He resigned in November, 1929, to farm the old homestead in Ontario. He returned to the Division of Botany and Plant Pathology and was stationed at Ottawa, where he worked as a Plant Disease Investigator for the summers of 1932, 1933, and 1934. Mr. Scott served successively as Junior Plant Pathologist, 1934 to 1937, Assistant Plant Pathologist, 1937 to 1941, Agricultural Scientist, Grade 2, 1941 to 1945, Agricultural Scientist, Grade 3, October through December, 1945, and Agricultural Scientist, Grade 4 from January 1, 1946 to the time of his death.

"Mickey," as he was familiarly known to his associates and to the seed growers throughout Canada, was chiefly responsible for devising and developing examination methods for seed-borne disease. He worked wholeheartedly with the Cooperative Vegetable and Cereal Seed Treatment Committees of the American Phytopathological Society in testing the newer fungicides on a variety of seed crops. Mr. Scott was keenly interested in the improvement of seed-treating machinery and its role in the production of healthy seed. He successfully urged the Canadian Seed Growers Association to adopt measures to ensure disease examination for all Foundation and Elite as well as certain Registered seed stock grown in Canada. His outstanding personal qualities enabled him to obtain team work not only among his colleagues in research but also in the applied field among the farmers and those engaged in the seed trade. He will be greatly missed by his many friends and associates for his genial smile and his frank ways.

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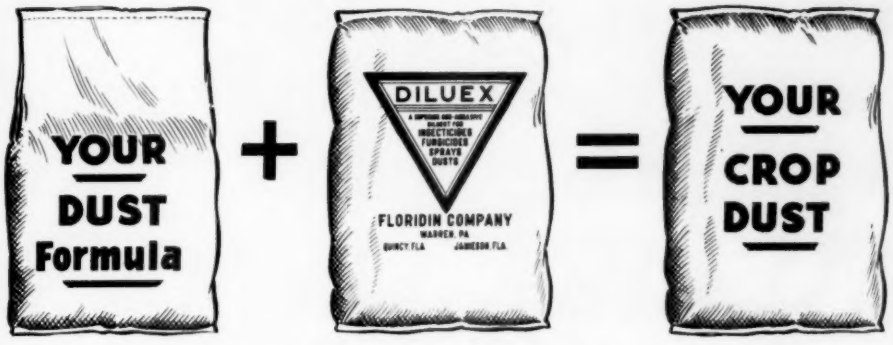

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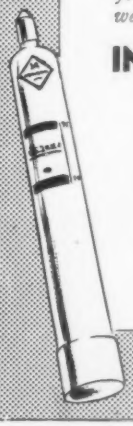

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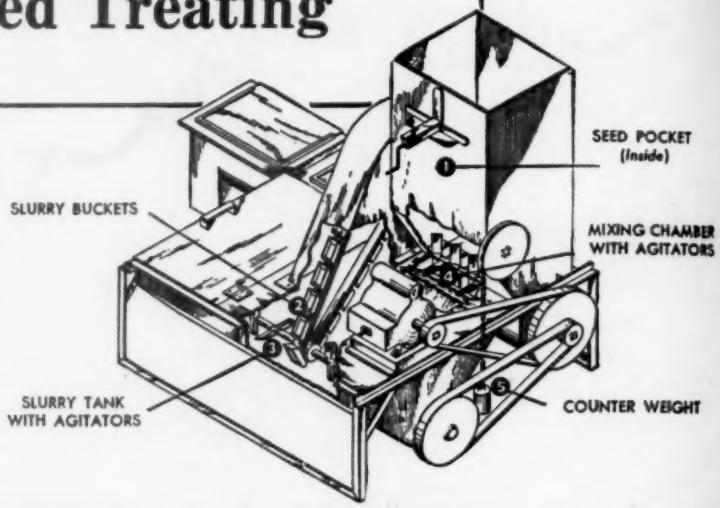

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